Linking genotype to phenotype:

A modelling framework for investigating individual and interacting mycelia

A thesis submitted in partial fulfilment of the requirements of the

University of Abertay Dundee

For the degree of Doctor of Philosophy.

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October 2006

I certify that this thesis is the true and accurate version of the thesis approved by the examiners.

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(Director of studies)

Abstract

The central question addressed in this thesis is the origin of complexity in individual and interacting fungal mycelia. A conceptual model identifying the minimal rule set responsible for complexity in terms of exhibited growth patterns is presented. The theoretical results replicate observed colony forms on agar and demonstrate that phenotype is sensitive to both environmental context and genotype. We show that the fungal phenotype may have its origins in the defining characteristic of indeterminate organisms, namely their ability to recycle locally immobilised internal resources into a mobilised form capable of being directed to new internal sinks. We show that phenotype can be modelled as an emergent property resulting from the interplay among simple local processes governing uptake and remobilization of internal resources, and macroscopic processes associated with their transport. The model demonstrates that the recycling process is crucial in soil-like environments where resources are limited and patchy. Theoretical analysis also shows that the recycling mechanism promotes optimised foraging depending on the resource quantity and quality, e.g. exploitative, when resource is plentiful and explorative when resource is scant.

The model is extended to represent fungal interactions between two distinct individuals by incorporating an additional inhibitor field. A range of observed interaction outcomes are replicated by modifying the parameters associated with the recycling apparatus only, based on the inhibitor field and trait values of the interaction process. The modelling framework is now in a form where an investigation into the origin of complexity underlying fungal community organisation can be undertaken.

Acknowledgements

I would like to thank my supervisory team Jim Bown, John Crawford and Nia White. Thanks to them for leading me down this interesting fungal path. Extended thanks to Jim for being a superb director of studies and helping to keep things in perspective (teaching and research). Thanks to John and Nia for their inspiration and endless enthusiasm for all things fungal and for making the work fun to carry out. Not forgetting Bill Samson who was originally on my supervisory team, I thank him for his advice and initial contribution.

Other people who have helped me throughout my PhD are Allan Milne, who would gladly talk to me (for hours!) about technical and historical computing matters for both research and teaching. Thanks also to everyone in SIMBIOS for their help and advice, at some stage or another, and providing a pleasant environment in which to work.

I will always be indebted to my family for supporting me, especially my Mum and Dad for being strong, encouraging and loving parents. Thanks also to Alasdair who has endured endless conversations regarding fungi and the Crank Nicholson method. Thanks to him for being there and providing me with many culinary delights.

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List of Abbreviations

CA Cellular Automata: Computer models that are spatially discrete and studied in Theoretical Biology and Mathematics. It consists of a grid of cells, each in one of a finite number of states. Time is also discrete, and the state of the cell at time t is derived from the states of the cells within a predefined neighbourhood. Every cell has the same rule for updating known as the transition rule.

CT Computed Tomography : originally known as computed axial tomography (CAT or CT scan) is an imaging method employing tomography where digital geometry processing is used to generate a three-dimensional image of the internals of an object from a large series of 2D X-ray images taken around a single axis of rotation.

CN Crank Nicholson: A finite difference numerical method used for solving partial differential equations. It is the average of the explicit and implicit method and imposes no stability criterion.

fBM Fractional Brownian Motion : Also known as a random walk process with memory dependence, the increments/steps are no longer independent i.e. there exist correlations between the events.

FTCS Forward Time Central Space: an explicit numerical algorithm used to solve Partial or Differential equations. It is based on centred difference approximations for space and forward difference approximations for time.

GA Genetic Algorithm: is a search technique used in computing to find true or approximate solutions to optimization and search problems.

GFP Green Fluorescent Pigments: is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light. In modified forms it has been used to make biosensors.

IBM Individual Based Model: Models in which there is an explicit representation of the individual and its associated characteristics/traits.

Chapter 1. Introduction – Biological Perspective

1.1 Fungi

There are an estimated 1.5 million species of fungi in the world (Hawksworth 1991), and fungi are so abundant they constitute an entire kingdom of Life, broken down into 4 groups based on reproductive mechanism. In spite of their abundance, they are largely invisible as they inhabit an opaque substrate; indeed, only 70,000 species are known. In addition to their prevalence, fungi are thought to represent the largest and oldest organisms on Earth. A single colony is growing in the subterranean world of a Michigan forest, and its spatial extent, weight and age are approximated to be 15 hectares, 100 tonnes and 1500 years respectively (Smith *et al.* 1992). It is the fungal growth form, often referred to as the mycelium that allows the potentially indefinite existence of this fungal colony. The fungal growth form is often referred to as indeterminate (Rayner *et al.* 1995) as unlike most determinate organisms fungi appear to have no programmed upper limits in their spatial and temporal extent. The fungal colony typically comprises a vegetative mycelium and a range of differentiated structures such as reproductive organs, (the mushrooms that are commonly seen beside trees in the forest in autumn), survival or resting structures (e.g. scelerotia) and transport structures (cords and rhizomorphs) for transporting resources. The mycelium is made up of hyphae (singular hypha) that have diameters ranging between one and thirty micrometers: these hyphae are the basic unit of the colony (Fig. 1.1). This thesis is focussed on mycelial fungi only. Additionally, some fungi can be unicellular, i.e. yeasts, and some may be dimorphic, i.e those that switch between unicellular and mycelial, although these are not considered here.

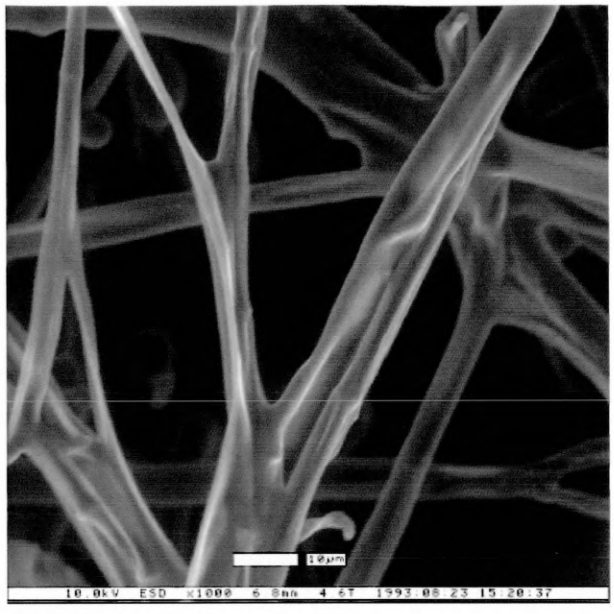


Figure 1. 1 branching of fungal hyphae from - www.ucmp.berkeley.edu/ esem/fungalfi.gif

1.2 Fungal Lifestyle

Many fungi pass successively through three phases: Colonisation and growth (vegetative growth), sporulation and dispersal (anamorphic phase) and reproduction (telemorphic phase) (Brasier 1999). This thesis is concerned with the colonisation and growth phase (i.e. production of vegetative mycelia) of fungi in general. During this phase of the lifecycle, all species of fungi carry out elementary processes including apical growth, branching, uptake, redistribution and recycling. These occur to varying degrees depending on the fungal species, as described in section 1.3. Further, the most significant interactions between fungi and the environment (both biotic and abiotic) occur during the vegetative phase (Boddy 1984) resulting in macroscopic colony morphs, termed phenotypes. As understanding the origin of individual and interacting mycelial phenotypes is a major component of this work it is sensible to focus on the colonisation and growth phase of development. Moreover, it is acknowledged that the incorporation of the other two reproductive phases in the first instance would introduce a degree of complexity that would preclude model development at this stage. This complexity is due to the myriad and sophistication of the reproductive mechanisms, on which the classifications of fungi are based.

1.3 Organisation and growth of mycelium

1.3.1 Growth

Hyphae are filaments that grow apically, extending from their tips, and, together with its capacity for branching this allows the mycelium to exploit substrate effectively due to its space filling capacity. For this growth to occur it is necessary that the hyphal tip is fuelled with resource that is subsequently synthesised into new hyphal wall material. Further, the nascent wall at the tip is required to be thin and plastic allowing extension of the hypha (Carlile *et al.* 2001) and the transport of enzymes. In time the sub-apical hyphal wall matures, during which it becomes thickend and rigid. The hyphae branch just behind the advancing tip at a distance characteristic of the species (Garret 1981). Initial branches produce new branches, but an orderly hierarchy of development is maintained. Hyphal tips at the periphery of the colony grow avoiding each other (negative autotropisms) forming a coherent network (Fig. 1.2a).The hyphae of higher fungi usually possess septa (cross-walls) that divide the hyphae into compartments; non-septate fungi are referred to as coenocytic. In septate fungi, the cross walls are usually perforated, possessing one or more pores and this allows the flow of cytoplasm containing eukaryotic organelles to flow through the fungal network (Fig. 1.2b). A fundamental distinction within fungi is the ability of septate hyphae to fuse with other hyphae of the same colony or species; such fusions are called anastomosis, and occur only when there is both a meeting of hyphal tips and those tips exhibit positive autotropisms by growing towards each other (Fig. 1.2a). Anastomosis facilitates the transport and redistribution of nutrients (Rayner 1995). Further, hyphal fusions play a fundamental role in the construction of fungal tissues and organs such as transport tubes (mycelial cords, rhizomorphs) and reproductive structures (mushrooms).

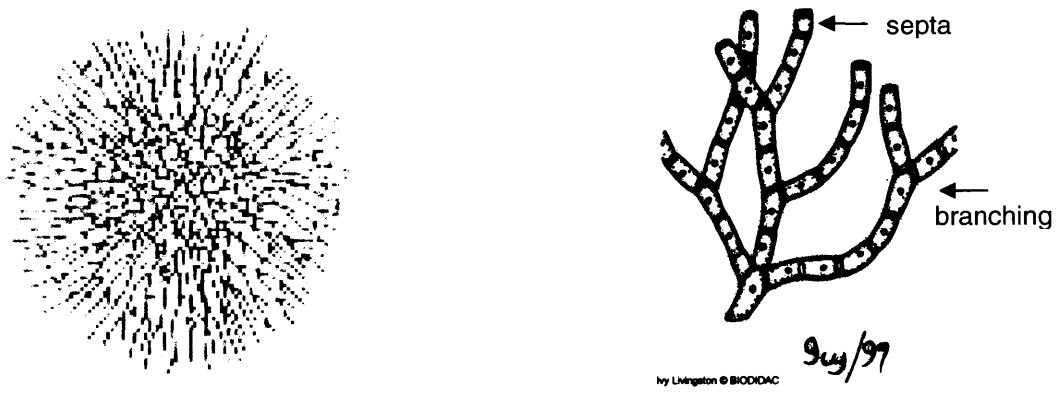


Figure 1. 2 a) circular colony showing positive (centre of the colony) and negative (periphery of the colony) autotropisms; b) Showing septa (cross walls) within the hyphae a) is reproduced from A.H.R. Buller's, Researches on Fungi 1971. b) from www.biodidac.bio.uottawa.ca

1.3.2 Uptake

In order for the mycelium to grow it must acquire resources from the environment and transport these resources in a suitable form to growing hyphal apices. Fungi increase the availability of external resources by releasing extracellular enzymes probably from the hyphal tip region into the environment (Walker and White 2005). These enzymes break down the complex organic resource into simpler compounds that can be absorbed by the hyphal network. Nutrient uptake occurs by transport across a semi-permeable membrane (Griffin 1994) that is thought to be located in the vicinity of the tip, as the older parts of the hyphal wall are rigid with considerable mechanical strength (Carlile 1995) and are thus less permeable. Experimental data regarding the localisation of nutrient uptake and enzyme secretion is extremely scant.However, Ruel and Joseleau (1991) identified that fungal enzymes are located in a sheath that mobilizes fungal enzymes to their substrate surface. Further, this sheath accumulated principally at the hyphal apex. Nutrient uptake occurs as the colony encounters fresh resource, and the mitochondria localised behind the apex supply energy (ATP) to support exchange across the membrane. Not all resources are accessible to all fungal species since the mycelium must release enzymes that are capable of degrading the resource. Therefore some recalcitrant resources may only be utilised by specialised fungi. The filamentous form of the colony allows efficient spread, tip initiation and uptake to effect maximum uptake of nutrients.

1.3.3 Redistribution

Fungi are sophisticated transport agents and nutrients, in the form of vesicles, are moved around the protoplasmic-filled hyphae to areas of the colony requiring investment (sinks). Investment can be in the form of apical extension (direction of growth), production of reproductive fruiting bodies, protective structures (sclerotia) and the production of enzymes and inhibitor compounds (Amir 1995). The exact driving forces of internal transport of nutrients are unknown (Watkinson *et al.* 2005; Read and Hickey 2001), although several mechanisms have been identified by Cairney and Burke (1996) and Olsson (1999). Passive translocation involves the absorption of nutrients by hyphae according to their requirement for growth in the region of uptake. Transport of nutrients within the networked colony then occurs by diffusion. In other cases, the nutrients may be taken up in excess of any local requirement and is termed active translocation. If nutrients are taken up far in excess of local need then this causes an osmotic difference that causes water to flow into the cell creating turgor (Cairney and Burke 1996). This results in flow of water and nutrients in the direction of least resistance. Finally, active translocation may also be carried out via cytoplasmic movements, in this case nutrients can be transported via the movement of organelles or by peristalsis of vacuole systems (Olsson 1999). The transport of nutrients within the mycelium allows a form of 'communication' among different parts of the colony. Thus the colony can be considered an integrated unit and is more than the sum of its individual hyphae. Events occurring within the mycelium cannot therefore be totally independent and the local environments occurring within the mycelial boundary cannot all be treated as entirely separate domains (Rayner *et al.* 1995).

1.3.4 Recycling

Fungi are constantly responding to the environment by reacting to, and governing, nutrient flow within the hyphal network. As well as investing in the areas of high metabolic activity, fungal colonies are also effective at divestment where necessary. Aged parts of the colony, i.e. those that have become insulated and rigidified and where the local underlying resource base has been exploited, are often recycled (mobilised) and emptied of protoplasmic contents. This recycled internal resource and biomass may be transported to younger parts of the colony or areas requiring other forms of investment (e.g. antibiotic production). This recycling allows the colony to essentially

reallocate itself (Fig. 1.3), degrading in the areas that have become redundant and growing into unexplored territory in search of new resources to exploit. Boddy (1993) state that there is reallocation of nutrients as well as biomass when mycelial cord systems encounter new resources. By effectively routing nutrients and mobilised (recycled) biomass to the areas of the colony in most need, fungi respond rapidly to changes in environmental context. For example exploitative growth when hyphae contact a new food supply is effected by mobilizing and reallocating resources to exploit the new food sources (Blackwell *et al.* 2005), and explorative growth when nutrients are scarce (Ritz 1995; Boddy 1999).

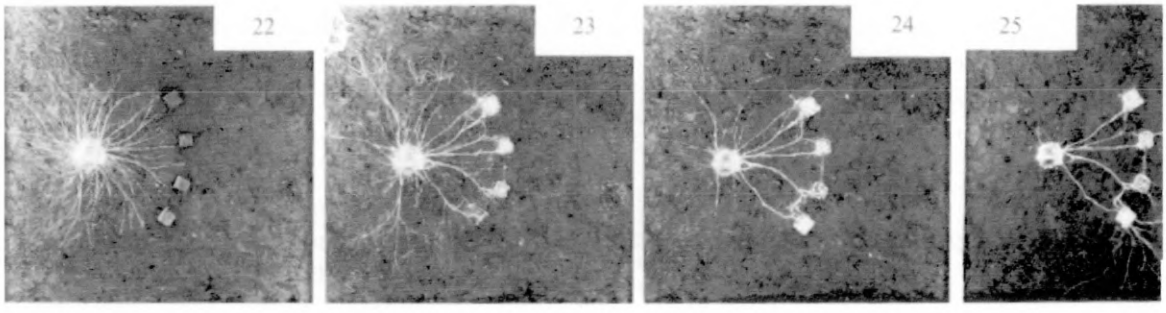


Figure 1. 3 demonstrating recycling of biomass, reproduced from Boddy 1993 (Mycological Research)

1.3.5 Non-local effects

The transport of internal nutrients and mobilised biomass is a non-local process, i.e. it occurs over a larger scale than that of the immediate environment, and can be attributed in some cases to diffusion alone (Ritz 2004). This implies that colony growth is not just a result of local processes. Olsson (1999) hypothesised the effect of different transport mechanisms, discussed in the previous section, on emerged mycelial density profiles of fungi growing on agar. The transport mechanisms are hypothesised to alter biomass profiles, confirming that mycelial distributions are a consequence of local and non-local processes. The whole mycelium may therefore be thought of as an interconnected, cooperative network where the local environment of one part may affect distant parts (Lindahl and Olsson 2004).

1.3.6 Mycelial Complexity

Fungi are versatile organisms capable of changing their form as a result of the environmental conditions; they appear to self-regulate by altering their internal partitioning of resource and are

thus capable of varying the balance between exploration and assimilation, conservation and redistribution of resources in response to the local environment (Rayner *et al.* 1995). A positive feedback effect occurs in the mycelium, resulting from the uptake of resource from the environment leading to an increase in biomass that subsequently increases uptake and is thus autocatalytic. Further, negative feedback, affected by the absence of nutrients and the transport mechanisms involved in the transport of nutrients within the network, counters the expansive drive of the mycelium. Furthermore, these processes (feedback and self-regulation) allow the fungal colony to self organise, an attribute of a complex system. The complex colony patterning arises from simple procedures that all 'biomass elements' undergo. The understanding of these simple procedures is key to understanding the origin of individual and interacting mycelial phenotypes.

1.4 Interaction with environment

Fungi interact with the environment and are affected by its physical, chemical and biological characteristics. This interaction occurs at the hyphal level and the result of these interactions is the phenotypic manifestation that influences the survival, resource capture and spread of the whole colony (Boddy 1984). Further, since a single fungal individual can potentially cover a large spatial area, the colony exposes growing tips in different regions to different environmental conditions. This results in different parts of the fungal colony operating under different modes (exploration, exploitation, sporulation) depending on its local environment. An example of such behaviour is diffuse versus directed growth of different parts of the colony in response to high or low nutrient availability (Fig. 1.3). Factors effecting the observed phenotypic form of the fungal colony include intrinsic genetic properties of the fungus (Ritz 2004), such as extension rates, branching mechanisms, transport mechanisms, recycling abilities, ability to form higher order structures, and the environment, where environmental features include biotic and abiotic elements such as physical habitat, microclimate, chemical and biotic heterogeneity.

1.4.1 Intrinsic genetic properties

The genetic identity of a fungal colony and its response to environmental conditions cause organisational patterns to emerge. Colony morphologies and growth rates in nature are often used as a basis for fungal identification (Brasier 1999). In fact some species form morphological

distinctive characteristics just by culturing on agar plates (Watanabe 2002). If fungi are grown under identical environmental conditions the differences in morphology can provide indirect information about the genetic differences among those colonies. Figure 1.4 below shows the range of colony morphologies for different species of fungi belonging to the same genus. Analysing colony forms on agar is useful for identifying genetically distinct individuals since fungi are readily replicated on cultured media and because of the ease with which key environmental components such as nutrients, pH or temperature can be controlled via the petri dish (Brasier 1999). Species growing on soil can also be identified by their morphologies (Fig.1.5), and this can often elicit the foraging strategy of the species: sparse growers often have long range foraging capabilities whilst dense growers have short range foraging strategies (Boddy 1993).

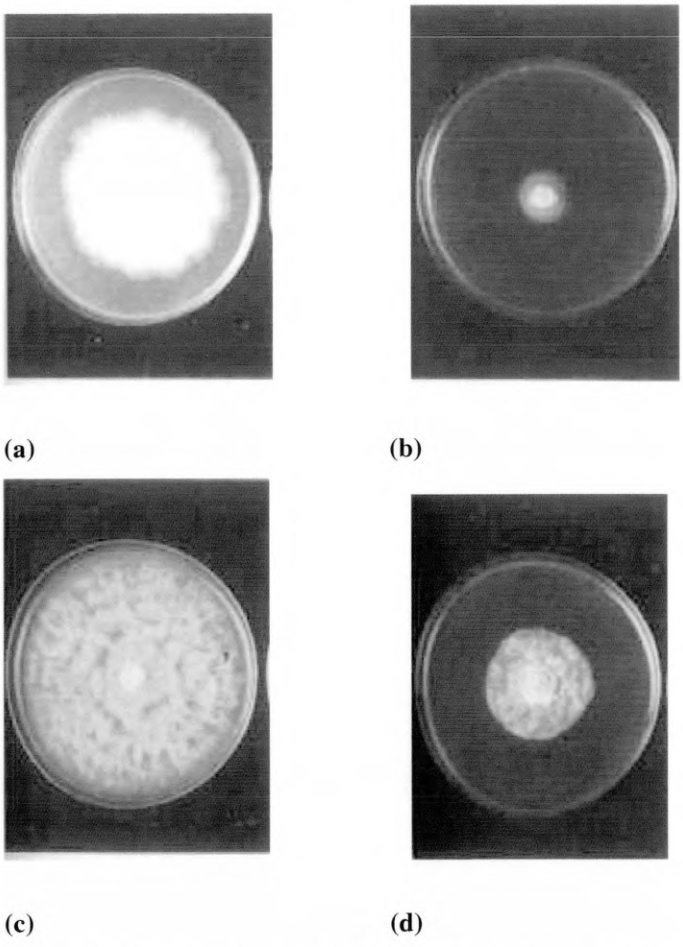
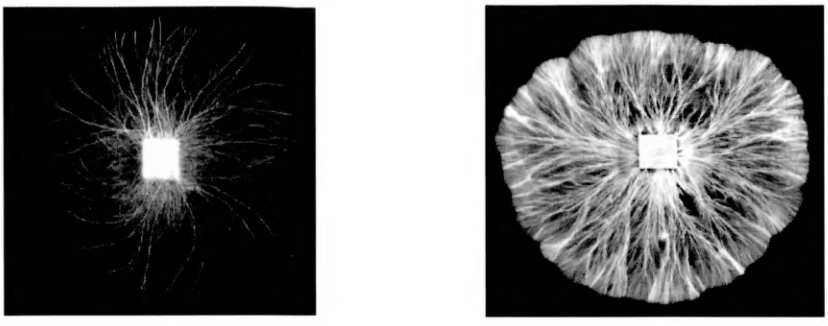


Figure 1. 4 Growth of four different species from the same *Phytophthora* genus, from Kenndey and Duncan 1995 a) *P. idaei* b) *P. cactorum* c) *P. citricola* d) *P. syringae*



(a) (b)

Figure 1. 5 Fungal growth of colonies (a) *P. Velutina* and (b) *H. fasciculare* on sterilised compressed soil from Watkinson et al., 2005

1.4.2 Environmental Features

1.4.2.1 Microclimate

Elements of the microclimate affecting colony growth and morphology include temperature, pH and water potential. Fungal growth is most obvious in damp conditions and most fungi grow best at moderate water potentials in the range 0 to –1 Mpa (Carlile *et al.* 2001). For most fungi, temperature variations exist in their growing environment but most have a lower temperature limit for growth, a few degrees above zero, and a maximum temperature for growth, between 30-40 °C. If nutrient conditions are satisfied most fungi can grow in a wide range of pH values, typically 4-7 (Carlile *et al.* 2001). These factors affect different species to varying degrees and in all of these cases there exist fungal species that have adapted to suit the most extreme and inhospitable conditions. These are termed extremophiles and some can exist in conditions of high acidity and temperatures (Wolfe 2001). Much of our understanding of the factors influencing fungal community structure and dynamics have been derived from wood and wood/soil systems. This has arisen from the importance of fungi in forest ecosystems and the simplicity of the experimental system for such a study. Many of these principles are extrapolated to soil systems which are inherently problematic to investigate.

1.4.2.2 Physical habitat

The physical structure of the habitat also plays a fundamental role in the organisational distribution of biomass and this structure is inherently linked to the growth medium. Here we consider only two of direct relevance to this thesis.

a) Agar

Agar is a complex polysaccharide derived from seaweed and when mixed with a nutrient source it provides a homogeneous resource distribution (in two and three dimensions depending on the thickness of the agar), which is a popular growth medium for the study of fungi. The advantages of agar systems are the ease with which they can be replicated, manipulated and controlled, e.g. across replicates all but one microclimate factor can be kept constant in order to determine its (isolated) effect on the growth of the species. Further, two-dimensional growth patterns are also easy to visualize. This type of experiment has been used to investigate aspects of ecological strategy (Brasier 1999). They have also been used in many experiments to determine the genetic properties of an individual as discussed above. Agar systems remove most of the complex environmental heterogeneities e.g. physical structure and heterogeneities with respect to resource and biotic elements, therefore providing a simpler environment with fewer complexities to obscure the identification of the processes responsible for a particular dynamic. The disadvantage of such systems is that they are far removed from the natural system and any results obtained from agar experiments cannot be readily transported into a more realistic environment. On agar fungi grow radially, extending from an inoculum, on, within and sometimes above the substrate. Agar allows control of initial levels of nutrients and this may contribute to our understanding of the complex relation between nutrient level (environment) and phenotype. For example, in Kennedy and Duncan (1995), altering the nutritional status of the agar gives rise to different colony morphologies of the same species (Fig. 1.6)

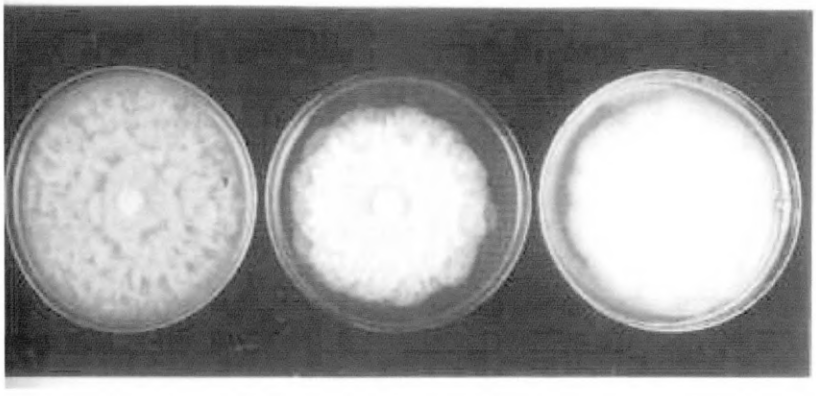


Figure 1. 6 Growth of *P.citricola* under different nutritional regimes (CV8A, MEA and SAMA) from Kennedy, D. and Duncan, J.M. 1995

b) Soil

Soil is a complex medium in terms of its physical, chemical and biotic framework and is a habitat for millions of organisms. In fact, in a handful of typical, healthy soil there are more creatures than there are humans on the entire planet, and kilometres of fungal threads (Wolfe 2001). The physical architecture of soil arises from an assemblage of variously sized solid phases, separated by a network of intervening soil pores. The pore space is occupied by air and the soil solution and most microorganisms live both within the pore space and also in the interior of the solid phases. Fungi can grow along the surface of a pore or grow across an air-filled pore space (Ritz and Young 2004), but they are essentially constrained by the pore network. Further, the basic architecture of the soil affects connectivity and tortuousness, ultimately affecting the foraging paths of fungi. Some of the architectural factors affecting colony growth and distribution include bulk density and water distribution of the soil. It has been observed that fungal extent is greater at higher bulk densities (Harris *et al.* 2003) and that water filled pores constrain fungal growth as they contain less oxygen (Otten *et al.* 1999). The main chemical component of soil is silica but soil is also composed of colloidal particles of clay and humus, which are chemically the most active. Essentially, soil consists of a corpus of organic material, and exudates from biota, which provide fungi with a supply of energy for growth and metabolism. The most typical substrates for fungi in soil are exudates from young, active roots that provide a nutrient solution of sugars and amino acids and dead plant tissue that fungi can decompose. A particularly well-known above-ground manifestation of fungal growth in soil are fairy rings. The fairy ring pattern results from the subterranean spread of fungi, growing slowly outward from a central point, and the reproductive structures (mushrooms) emerge

above-ground on the perimeter of the colony, where the most active and healthiest part of the colony is located. More typical below-ground fungal growth has no characteristic pattern, unlike the circular colony forms on agar, and it is very much constrained by the physical architecture. Fungi grow as threadlike strands along or bridging macropores and mycelial density varies greatly over small distances (Ritz and Young 2004), Fig. 1.7. Densities vary as a result of the distribution of nutrient sources and moisture, and this has implications on mycelial morphology and organisation.

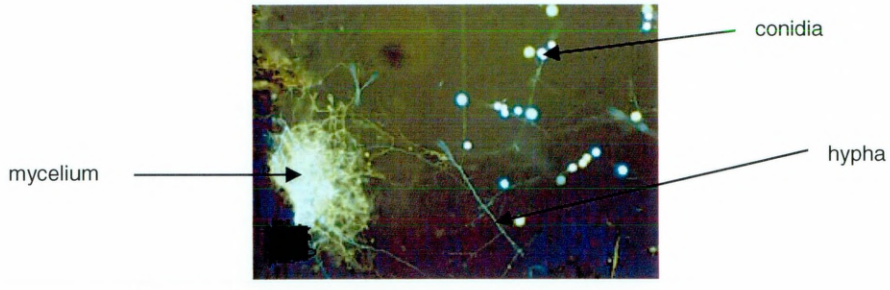


Figure 1. 7 Unidentified mycelium growing in soil pore, visualised in thin-section of undisturbed pasture soil, stained with Fluorescent Brightener 28. The thread-like strands are the hyphae (collectively the mycelium) and the bright circles are conidia from Ritz and Young 2004

1.4.2.3 Distribution of resources

The distribution of nutrient resources has a large impact mycelial organisation (Griffith and Bardgett 2000). When fungi encounter a new resource it is exploited effectively, resulting in dense mycelia that utilise that underlying resource. Once this resource has been exploited, sparse growth continues from the exploited resource until a subsequent resource is found and new investment occurs. Fungi must be capable of making efficient connections among resources and these connections set up source-sink relationships. The nutrients obtained from the source can be transported to various sinks at different locations within the colony, for example towards the direction of growth, reproductive structures and local combat areas (Ritz 2004). Finally, the presence of biotic competitors will effect mycelial development in environments such as soils and agar. In a soil environment fungal-fungal interactions are a common occurrence as it is highly populated by different fungi, thus the outcome of competition is important in shaping mycelial distribution.

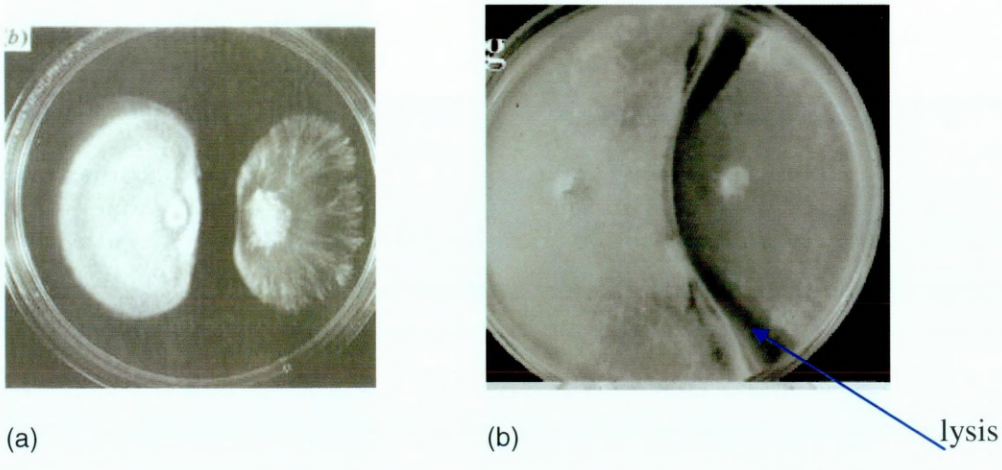
1.4.2.4 Fungal Interactions

Fungal interactions may be categorised into those among members of the same species, i.e. intraspecific, and those among members of different species, i.e. interspecific. There have been many mycelial interaction studies on agar (White *et al.* 1998; Boddy 2000; Cooke and Rayner 1984; Rayner and Webber 1984; Sturrock *et al.* 2002) due to the ease of observation of fungal individuals and the ability to determine the underlying mechanisms responsible for the interaction outcome. An example of hypothesised mechanisms for combative interactions are mediation at a distance and contact (Rayner and Webber 1984). Agar systems have also been successful in demonstrating the range of interaction outcomes possible between fungi.

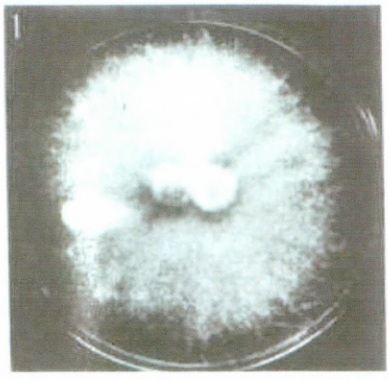
Gaining nutrients in agar and wood systems is intimately related to gaining access to space and to competition and can therefore be categorised into primary and secondary resource capture (Boddy 2000). Success at primary resource capture is associated with fast extension rates, good dispersal mechanisms and the ability to utilise a number of different and simple resource types as this ensures first access to the resource. These fungi are said to possess *R*-selected ecological strategies and are associated with an ephemeral existence (Boody 2000, Rayner 1999). Success at secondary resource capture involves gaining access to an already colonised resource by elimination of the competitor through combat; these fungi are often referred to as *K*-strategists and are associated with a persistent existence (Rayner 1999).

The mechanisms of combative fungal interactions include antagonism at a distance, hyphal interference, mycoparasitism and gross mycelial contact. These result in intermingling of species, deadlock, engulfment or replacement (Stahl and Christensen 1992; White and Boddy 1992). Often one of these interaction outcomes can be a prelude to another, e.g. engulfment normally occurs before replacement (Rayner and Webber 1984). Antagonism at a distance involves the release of a diffusible or volatile compound (Boddy 2000) that inhibits the growth of a fungal species. This can result in the replacement of one species by the other or mutual inhibition resulting in deadlock. Deadlock is often an outcome of fungal interactions on agar (Fig. 1.8a). Hyphal interference occurs

when non-self hyphae are in close proximity and is mediated through a diffusible metabolite. Upon detection of non-self hyphae, the hyphae of one or both colonies may degenerate often referred to as lysis. Mycoparasitism occurs upon contact and recognition of a host, subsequently growth over the host occurs from which nutrients can be obtained biotrophically or necrotrophically (Boddy 2000). Gross mycelial contact occurs when both fungi grow into one another and is often followed by a change in colony morphologies resulting in barrages, sclerotia, pigmentation change and redistribution of mycelium. In some cases if the hyphae of two fungal individuals are somatically compatible, usually self pairings, intermingling of hyphae will occur forming one dense mycelial mat (Reaves and Crawford 1994) and this is a neutralistic interaction. Interaction outcomes are sensitive to microclimate (water potential, temperature and pH), nutritional status (size and quality of resource), inoculum size (White et. al 1998) and a change in one of these factors can alter the interaction outcome. For example in environments with high nutritional status the interaction outcome of two fungal species can be deadlock. The same individuals but with a low nutritional status results in intermingling of hyphae (Stahl and Christensen 1992). Interacting fungi seem to reallocate and redistribute resources within the mycelia but experimental evidence of how they are reallocated is scant (Watkinson *et al.* 2005). Understanding how resources are allocated within and among interacting fungi is crucial in understanding how mycelial distributions of interacting fungi emerge. Fig. 1.8 below represents deadlock, replacement with lysis and intermingling.



(a) (b)



(c)

Figure 1. 8 a) and c) are from Rayner and Webber (1984) demonstrating deadlock and intermingling. Figure1.8 b) is from Boddy (2000) and this shows replacement and lysis.

Interactions between higher fungi (e.g. Basidiomycetes) have been carried out on wood and soil; these result in the same interaction outcomes as described above (Boddy 2000). There have been fewer studies of fungal interactions in soil but this is an acknowledgement of the difficulty associated with such a study. First, the mycelium is embedded within the opaque soil substrate therefore the spatial domain and distribution of individuals is difficult to determine. Second, there may be confusion regarding the limits of the interacting fungi because of the ability of hyphae to anastomose. Third, most experiments require replicates in order to obtain an average outcome or to determine results at various time points. Trying to replicate a given soil environment with the same porous structure i.e. identical intricate details of pore channels and throats ranging over scales together with chemical/nutrient distributions is impossible with today's technology.

1.5 Ecosystem Functional Roles

The dynamics of fungal colonies are central to the efficiency of forestry and agricultural system functioning. Fungal roles include saprophytes, mutualistic symbionts, pathogens, protection against pathogens and habitat engineers that are capable of mobilizing nutrients and thus affecting the physio-chemical environment (White 2003). The activities of fungi sustain life both above- and below -ground and their ecosystem roles are discussed below.

1.5.1 Decomposers and recyclers

Basidiomycetes fungi are responsible for the decomposition of organic matter such as woody debris and ultimately the mineralization of the nutrients contained within it. The mean value of the amount of plant remains deposited annually in the forests is 2 tonnes per hectare, and a quarter of this is in the form of woody remains that are degraded almost exclusively by fungi (Carlile *et al.* 2001). These fungi often produce transport structures (cords or rhizomorphs) that form connections between islands of woody resources allowing reallocation of resources within an extensive fungal network. Movement of nutrients (e.g. phosphorous) between these woody islands has been measured up to 0.75 metres, (Olsson and Gray 1998). Further, basidiomycetes are good colonisers and therefore provide competition helping to prevent invasion of the woody remains by other fungal parasites. Moreover, this network is an ecological compartment for immobilization of nutrients and some nutrients are 'leaked' out during the nutrient mineralization process. This leakiness is a result of inefficiencies of absorbing mineralized nutrients making available these nutrients to other organisms. All resources are ultimately released upon death and decomposition or through grazing by invertebrates (Dighton 2003).

1.5.2.Maintaining plant primary production

90% of approximately 248,000 species of higher plants on our planet have a mutual relationship with fungi (Allen 1993). In fact the majority of plant species could not exist within the natural environment without this symbiotic association between plant roots and mycorrhizal fungi. This partnership influences uptake of mineral nutrients from the substratum into plants to be used for plant biomass production (Dighton 2003). These fungi are also vital in protecting the host plant from pathogens (Newsham *et al.* 1995). The importance of mycorrhizal contributions to primary production in forested ecosystems is demonstrated in Vogt *et al.* (1982). This study showed that although mycorrhizal fungi contribute only 1% to total ecosystem biomass, the percentage of net primary production by mycorrhizal fungi was 14-15%. Mycorrhizal fungi are essential for survival of plants and trees during periods of water and nutrient stress. Fungi have a functional advantage over plants, as their hyphal diameters can be as small as 1–2 micrometers whilst the finest plant root is 20-30 micrometers. Hyphae can therefore access the smallest crevice in the soil and they

are more efficient at mining the soil for resource and uptaking nutrients that could not otherwise be exploited by a plant. Thus a plant's capacity to exploit a patch of soil expands tremendously with the prolific growth of its subterranean fungal partner (Wolfe 2001). For comparison, in 1 cubic centimetre of soil there would be a few centimetres of root and root hairs; however, the length of hyphae could be kilometres (Wolfe 2001).

1.5.3 Pathogens

Although some fungal species improve plant growth and productivity there are many cases in which the fungus is harmful to the plant, i.e. a pathogen. The classic examples of significant reduction in plant performance due to fungal pathogen attack is the devastating potato blight in Ireland in 1840's, elm and oak decline in Europe (Brasier 1996) and the decline of chestnut trees in North America (Dighton 2003).

1.5.4 Protection against pathogens

Some species of fungi can be used to prevent pathological symptoms caused by pathogenic fungi (Wakelin et *al.* 1999; Whipps 2004). For example the natural microbial population, including fungal species, of certain soils suppresses the development of wilt diseases caused by vascular wilt fungi such as *Fusarium oxysporum* (Doohan 2005). There are several commercial bio control agents that counteract disease cause by fungal pathogens, and these include: AQ10 Biofungicide to prevent mildew of many fruits and vegetables; Biofox C which prevents fusarium wilt of basil; cyclamen and tomato and Soilgard which prevents root rot of many plants caused by Rhizoctonia Solani (Doohan 2005). Finally, Duchesne (1994) summarises studies that have demonstrated that ectomycorrhizal fungi, a subclass of mycorrhizal fungi, suppress pathogenic disease of seedlings. This disease protection has been attributed to several active mechanisms including: antibiosis, the antagonistic association between an organism and the metabolic substances produced by another; the production of antifungal substances by the plant roots as a result of the interaction between plant roots and ectomycorrhizal fungi and a physical barrier effect caused by the fungal mantle around ectomycorrhizal roots (Duchesne 1994).

1.5.5 Improving soil tilth

As fungi grow through soil they apparently improve soil aggregation by hyphal binding and by exuding production of polysaccharide glues thus improving aeration and water flow through the soil pore network. At small scales spatial extension of hyphae through soil can cause a mechanical disturbance (Ritz and Young 2004) providing cracks that other microbes can live in, thus sustaining bacterial populations in soils. Additionally, the role of fungi in the formation and stability of soil solid phases by binding organic and mineral material in soil is of fundamental importance to both the fertility and stability of soil (Dighton 2003). Further, the nutrient component of soil for plants is derived partly by fungal decomposition of dead plant and animal remains. If fungi did not decompose such remains the complex organic components and mineral nutrients could not otherwise be recycled back into the ecosystem.

1.6 Other roles of fungi

Fungal fruiting bodies (mushrooms) have to resist infection and decay before sporulation is achieved. They do this by sweating out exudates or secondary metabolites and these exudates inhibit specific bacteria. These exudates can be studied and chemically similar compounds can be pharmaceutically synthesised and used to prevent infection in human beings. Antibiotics used in medicine that are obtained from fungal organisms include penicillin, fusidic acid, lovastatin, mevastatin (Murphy and Horgan 2005) to name but a few. Production of economically important industrial commodities such as miso, quorn™ and fermented beverages relies on the exploitation of fungal activities and metabolism (Walker and White 2005).

1.7 Applications of knowledge of understanding fungal systems

Evolution has resulted in increased diversity but, due to human intervention fungal and other species are being lost before they can be identified (Berry and Gleeson 2005). As fungal generations progress through a habitat soil depth, moisture and quality increases, enhancing the carrying capacity of the environment and diversity of its members (Stamets 2005). It has been recognised recently that strategies that maintain fungal diversity should be encouraged, as a loss in

fungal diversity may have severe implications for ecosystem health and functioning because fungi play an important role in determining the balance of above- and below- ground populations (Swift 2005). By maintaining a diverse soil fungal community this will ensure maximum recycling of recalcitrant organic matter, maximise plant community productivity (Bever *et al.* 2001), and carry out the important role of conditioning the soil. It is not entirely clear what effect a change in fungal community structure would have on primary plant production, nutrient recycling and soil quality. Further, it has been acknowledged that biodiversity should be conserved, as it is highly probable that there exists potential medical breakthroughs in as yet undiscovered fungal species (Hawksworth and Rossman 1997). In order to maintain diversity one has to understand how the system works and identify the mechanisms promoting diversity. Moreover, an understanding of the factors influencing community structure and their degree of influence can be applied in the areas of biocontrol and bioremediation.

1.7.1 Biocontrol

Interest in the interaction between pathogens and biocontrol fungi has led to the use of ectomycorrhizal fungi as biocontrol agents (Whipps and Davies 2000; Deuchesne 1994). There are a variety of fungal species and isolates that have been examined as biocontrol agents, but *Trichoderma* are the most common, perhaps reflecting their ease of growth and wide host range (Whipps and Davies 2000). *Trichoderma* have been used in many commercial biological control treatments such as Supresivit, PlantShield and T-22 which are used to control soil-borne fungi causing rot (Doohan 2005). A recent example of fungal bio control includes the use of *Trichoderma* to inhibit the *Rhizoctonia solani* pathogen that infects a number of crop plants such as the aubergine and pea plants (Lewis *et al.* 1998). Biological control is not a new phenomenon and is merely the exploitation of a natural indigenous protection of live trees by native saprophytic and endophytic communities. These communities thrive upon the tree's abundant dead organic matter, guarding against invading parasitic fungi (Dighton 2003). Biocontrol strategies can be exploited to manage fungal pathogens attacking our crops, i.e. non-blighting fungi may be the best defence

against blighting fungi (Stamets 2005). By pre-filling (prescribing) the susceptible niche with a chosen species an invasion by blight fungi can be forestalled or prevented.

1.7.2 Bioremediation

Fungi have been healing and steering our ecosystems on their evolutionary path for centuries. The healing properties of fungi can be exploited to remedy ecosystems suffering from toxic build up (bioremediation) and poor nutrition. This has significant advantages over current methods such as burning, removing or burying toxic waste (Stamets 2005), which do not solve the toxicity issue and do nothing to improve the ecological niche. Prescribed fungal communities can be used to denature toxins and absorb heavy metals to restore the soil quality allowing it to continue to support plant life (Fomina *et al.* 2005). Some fungi are adept molecular dissemblers, breaking down many recalcitrant chemicals into smaller less toxic chemicals by the enzymes they exude (Gadd 2004). Heavy metals can be removed from the land by channelling them to the fruiting body for removal. Mycoremediation can be achieved by mixing mycelia into contaminated soil, by placing mycelial turf mats over toxic sites (Stamets 2005), or by spraying the contaminated environment with pre-inoculated wood chips. A potential use of mycoremediation is described in Stamets (2005) and involves using hair to mop up petroleum spills or leaks. Hair naturally absorbs oil, which can then be saprophytised by fungi. This has potential uses for mopping up and degrading petroleum from oil spillages at land and sea.

1.8 Towards managing fungi

Understanding and protecting the health of our environment is dependent on our understanding the roles of complex fungal individuals and communities. Central to this is a need to understand how individual and interacting fungi grow and develop in their natural environments. We must be fully aware of the implications of releasing a fungal species into a target environment, whether for biocontrol or bioremediation. We must learn from our mistakes and understand all facets of a new technology before it is heavily exploited. It is now known that the extensive use of chemistry in the 1960s (plastics, alloys, pesticides and petrochemicals) has levied a heavy toll on the biosphere,

although in their time they were considered the fruits of science. Before applying any new technologies we need to understand fully how they will interact and evolve with its environment. Modelling is often a useful tool to help gain an understanding of a system so that this understanding may be applied in a predictive sense (White and White 2005). Ultimately here, we must be able to assess the effect of adding a particular species to targeted problem populations, avoiding epidemic disease or monopolization of the niche by the added species, or manipulate the environment to affect changes in the microbial community and manage the process.

Chapter 2. Introduction – Modelling Perspective

2.1 Modelling Paradigms

Scientific models fall into two categories: Those that make predictions about the system under study (empirical models); and those that aim to further our understanding of the modelled system (investigative models). The modelling approach undertaken is driven by the underlying research question(s). The questions, often encapsulated in hypotheses, arise from the need to understand the origin of observed patterns in data. The model is implemented and the hypotheses addressed by comparing model patterns (results) against observed patterns. The model is modified, and this typically involves either a data fitting exercise or a fundamental model reformulation, until a reasonable match between model and observed results is obtained.

Empirical models are based directly on observed data sets (Brown and Rothery 1993). These patterns are found statistically or, less commonly, by searching state space using Principal Component Analysis, Genetic Algorithms and Neural Networks. A formulated procedure, usually included within the functionality of a statistical computer package, is used to solve the model by finding a function that fits the data. Although the empirical model cannot be used to explain or understand the origin of the patterns exhibited by the system, the model can predict behaviour where missing data exists by extrapolating and/ or interpolating the fitted function. These models require substantial datasets to support the statistical analysis and the predictive output is not generic, i.e. it is constrained to the particular state space from which it was derived and extrapolations far from the observed data may be inaccurate.

Investigative models describe assumed processes of the model using mathematical relationships and try to establish relationships among attributes of a system. The complexity of the mathematics used to formulate these processes affects the subsequent analysis of the model. If linear mathematics is used in the construction of the model then analytical solutions may be found. The advantage of analysis is that solutions are exact and general. Given the form of the solution one is

able to determine the solution for any numerical values of the initial conditions. Classical mathematics has been concerned with linear mathematics for a sound pragmatic reason: analysis of nonlinear mathematics could rarely be undertaken using paper and pencil. Classical models ignored awkward nonlinear terms, and, if the effect of these terms was small, the models produced reasonable approximations. A good example of this is the swing of a pendulum; classically the nonlinear sine term was ignored providing a theory for small swings only. From resolving forces acting on the pendulum (see Brown and Rothey 1993) we obtain:

$$mR\frac{\partial^2 x}{\partial t^2} = mg\sin x \qquad 2.1$$

where R = radius (length of string suspending the pendulum), m = mass of pendulum, g = force of gravity, x = angle the pendulum makes with the vertical and

$\frac{\partial^2 x}{\partial t^2}$ = angular acceleration of the pendulum. The above equation (2.1) is derived from F = ma and can be simplified to:

$$\frac{\partial^2 x}{\partial t^2} = -c\sin x \text{ where } c = g/R. \qquad 2.2$$

Analytical solutions can be found easily if we linearize the system, i.e. remove the nonlinear sin x term. Conveniently, for small angles of x, ($|x| << 1.0$) then sin (x) $\approx$ x. Therefore for small swings only:

$$\frac{\partial^2 x}{\partial t^2} = -cx \text{ where } c = g/R \qquad 2.3$$

Assuming the pendulum starts from rest ($x = x_o$) at t=0, and from integral tables the solution takes the form:

$$x = A\sin\sqrt{ct} + B\cos\sqrt{ct} \qquad 2.4$$

Applying the initial conditions we can determine the values for the arbitrary constants A and B. At t = 0:

$$x(0) = A\sin(0) + B\cos(0) = B \therefore B = x_o \quad 2.5$$

$$\frac{\partial x}{\partial t} = \sqrt{c}A\cos(0) - \sqrt{c}B\sin(0) = \sqrt{c}A = 0 \therefore A = 0 \quad 2.6$$

Hence the solution is:

$$x = x_o \cos\sqrt{ct} \quad 2.7$$

This provides us with a general formula to determine the amplitude of the pendulum at any t from any initial starting amplitude, x_o. This model is an oversimplification of the real system, in which the pendulum would suffer from drag or friction and would eventually cause it to stop swinging and it is limited to small swings only. For large swings there are no analytical results that are easy to formulate and therefore numerical solutions are often employed. For larger swings we end up with 2.8 and an analytical solution of this expressed in terms of elementary functions is not possible.

$$\frac{dx}{dt} = 4\sqrt{\frac{2g}{L}}\sqrt{\cos x - \cos x_o} \quad 2.8$$

Given most physical systems in nature are inherently nonlinear, e.g. cell processes, the Earth's weather system, plant and tree forms, then most models describing natural phenomena must also be nonlinear e.g. Navier stokes equations, Boltzman transport equations and fractals. Therefore the use of linear mathematics to describe biological processes is limited. With the advent of the computer, and consequent computer-based numerical solutions, nonlinear terms could be incorporated into models and the solution for any given initial condition obtained at the expense of an exact and general formula. These analytically intractable mathematical models still have solutions but in order to obtain these solutions numerical analysis or methods must be used. Unlike analytical solutions, numerical solutions are not generic and the solution obtained is dependent on the initial conditions. For different initial conditions a different solution is obtained and how these solutions are related is not known. Numerical solutions, i.e. those obtained from numerical methods, are approximations to the continuous model and are obtained by discretizing space and time. A numerical solution (simulation) divides up the spatial and temporal domain into discrete segments called the mesh spacing, and this is a discretized grid. The mesh spacing has implications on the accuracy of the approximated solution. The smaller the value for the spatial and

temporal step the more the numerical solutions approximate the continuous equation, resulting in a more accurate result. However, by setting a small spatial and temporal step the computation may not be practical, as it takes too long to solve. The numerical results are, therefore, at best as good as the mathematical model, since the numerical method results are an approximation of that mathematical model.

Simulations are iterative, constantly re-computing the underlying equations at each time step simulating the dynamics of the modelled system. Choosing the correct numerical scheme for the given problem is crucial as all numerical schemes have limitations. For example, some may cause instabilities and others may not be computationally practical as a large number of spatial and temporal steps are required in order to obtain an accurate solution as discussed.

For nonlinear systems, qualitative analysis may also be carried out to determine the qualitative rather than quantitative nature of the solutions (Brown and Rothery 1993). For example, it may be useful to find the general conditions for which the model has a stationary equilibrium or supports travelling waves. For local stability analysis, small perturbations to the parameter values about the equilibrium are applied and the effect determined. If the system is attracting then the same solution, i.e. the solution at the equilibrium, dominates the behaviour when the initial parameter values are close to the initial values of the equilibrium point. For any problem it is possible to find and classify possible equilibria according to their stability properties. This informs one of the general long-term behaviour of the solution e.g. whether it is stationary, periodic, aperiodic or chaotic. Another type of qualitative analysis is the identification of any travelling waves. Nonlinear systems may only allow certain wave profiles to propagate, each one with its characteristic velocity (Grindrod 1991). A travelling wave solution has the form $f(x - ct)$ and the shape, f, of the solution does not change where speed c is constant for a given initial condition. Travelling waves have been widely studied in many areas and represent the movement of some quantity or spatial pattern that does not change shape and moves at a constant speed such as an advancing front of invading populations and the rate of wound healing (Sherratt and Murray 1990). Studying travelling waves allows us to identify

the initial conditions and parameter set that give rise to travelling waves with characteristic velocities. Experimental data can be used to verify the result.

Although challenges exist when using numerical methods, unlike analytical methods they make it possible to theoretically model and simulate real-world phenomena. In some systems both types of models (numerical and analytical) are explored and compared by constructing a simplified approximation to the detailed numerical model that may be analysed analytically (Hiebeler 1997). Models by Richards *et al.* (1999) use a combination of numerical and analytical approaches to investigate a host-disease system where a population is subject to rare disturbance events. The analytical model predicts that if the host population is monomorphic and at equilibrium then a mutation can invade the population if it leads to an increased birth rate or a decreased mortality rate. The numerical models were used to investigate the effect of localised interactions, e.g. the numerical models differed in how they assume individuals distribute themselves within their habitat. These showed that spatially localized interactions can have a pronounced effect on the evolutionary dynamics of the system.

The adopted mathematical model should be based on as thorough an understanding as possible of the problem domain and should incorporate key features only, including detail that is relevant and omitting irrelevant details. Determining what features are relevant and irrelevant to the processes of the mathematical model is non-trivial and prototypes are often used to guide this process (Budgen 2003). A prototype is produced quickly and used to help determine requirements of the system, enabling developers to see exactly what functionality the system can provide and its limitations. Once the minimum set of processes (requirements) has been determined that describe the underlying system, the quality or worth of the model relies on its ability to reproduce the behaviour of the real system and when necessary, it is reformulated via another prototype development. This is often an iterative process requiring several prototypes that evolve from the previous version. Note Appendix B details the prototypes that led to the development of the theoretical model

presented in Chapter 3. The formulated mathematical model is then solved analytically or numerically and investigations are carried out to address the hypotheses of the system.

Models, whether analytical or numerical, can be classified by how the equation set (mathematical model) is developed and implemented. Common classifications relevant to biological and ecological modelling are: Mean-field, Individual-based, Process-based and Cellular Automata models (Wolfram 2002; De Angelis *et al.* 1992). These classifications are not mutually exclusive and there exists a degree of overlap between classifications, e.g. under certain conditions Cellular Automata may be considered a subclass of Individual-based models (Reynolds 1995) and a model may be considered both Individual-based and Process-based (Pachepsky *et al.* 2001).

2.1.1 Mean-field Models

Mean–field models average over space any individual characteristics and simulate the changes to the mean characteristics for the whole population (Grimm and Railsback 2005). These models are described by linear or nonlinear differential equations in which a single variable represents population density. They have had a great influence on theory in ecology and the most famous is the Lotka Volterra predator-prey model (Brown and Rothery 1993). The Lotka Volterra predator-prey model describes the long-term relationship between two well-mixed, mean population abundances of a predator and its prey species over time. The numerically derived solutions, due to the nonlinear model, demonstrate that both populations coexist with the predator population lagging behind the cyclic variation of the prey population. The two populations do a waltz through time (Trefil 2002), a change in one generating a change in the other. The prey population, H, changes due to a growth rate, rH, and a rate governing the probability that a predator, P, will encounter prey, AHP. The predator population changes due to a natural decline in the absence of prey, $-qP$, and a rate governing the probability that a predator will encounter prey, BHP. This results in the following population model:

$$\frac{dH}{dt} = rH - AHP$$

$$\frac{dP}{dt} = -qP + BHP$$

Here, r is the growth rate of prey in the absences of predators and q is the rate of decline of predators in the absence of prey. The constants A and B are rates at which predator-prey encounters remove prey from the population and the rate at which these encounters allow predators to add to their population respectively. From these equations we can see that the form of the solution is independent of individual variation and space. As extensions such as space and interactions among individuals are added, mean-field models that were analytically tractable become intractable and need to be analysed numerically.

It is now widely accepted that space and individual variation have dramatic effects on population behaviour (Levin 1992; Goldwasser *et al.* 1994; Tilman 1994). Consequently, the inclusion of these is required if we wish to ensure our models are sufficiently detailed and realistic to reflect the real world and be applicable to specific problems in managing the natural systems discussed in Chapter 1. Modern theory construction should not be bound by the limits of analytical mathematics.

2.1.2 Individual Based Models

There is much debate about the definition of an Individual-based model (IBM) (Grimm and Railsback 2005). Typically IBMs differ from traditional analytical approaches in two ways: all individuals are different, and the environment does not affect all individuals equally. An IBM tracks characteristics, or traits, of an individual and a degree of variability exists among individuals (Lomnicki 1999). The traits parameterise the processes and interactions of an individual with its environment and other individuals. In an IBM the behaviour of each individual is simulated explicitly. One important characteristic of such models is that they do not specify any global, population level rules such as exponential population growth. If exponential growth of the population arises, it does so as a result of emergent interactions amongst individuals, e.g. uptake, metabolism and competition. The organization levels (populations, communities, ecosystems) are viewed as complex systems that arise from the interactions of the individuals through adaptive behaviour (Kreft *et al.* 1998).

In addition to individual variation IBMs support an explicit account of space. This is important as it has been widely accepted that spatial configuration affects the macroscopic growth of bacterial and fungal colonies (Kreft *et al.* 1998; White *et al.*1998) and community dynamics of organisms (Tilman 1994; Goldwasser *et al.* 1994). The distribution of a species over space is a fundamental and inseparable aspect of its interaction with the environment. Individual based models are thus used to explore the effects of individual interactions and variation on community dynamics, in a spatially explicit environment. In summary, the main features of an IBM include:

- The individual as the fundamental component of the model;
- an environment in which interactions among individuals occur;
- the behaviour of the individual that is controlled by a set of characteristics (traits).

The individuals may be mobile or immobile, as determined by the problem domain, and a global pattern emerges as a consequence of interactions among individuals. IBMs have been used in many areas such as bird, bacterial colony, plant, plankton and crocodile dynamics (Reynolds 1995; Kreft *et al.*1998; Pachepsky *et al.* 2001; Batchelder and Williams 1989; Richards 2004 respectively). One simple example of an IBM is Reynolds's Boids program that demonstrates coordinated bird flocking. Each virtual bird (Boid) is described by three simple steering behaviours (processes) that describe how an individual bird manoeuvres based on the positions and velocities of its nearby flock mates. The following diagrams Fig. 2.1- 2.3 illustrating the main features of the model are extracted from http://www.red3d.com/cwr/boids/:

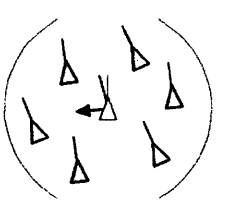


Figure 2. 1 Separation: steer to avoid crowding local flock mates. This avoids any direct collisions amongst other Boids.

Figure 2. 2 Alignment: steer towards the average heading of local flock mates. This entails adjustment of each individual Boid's velocity to match up with the rest of the flock's velocity.

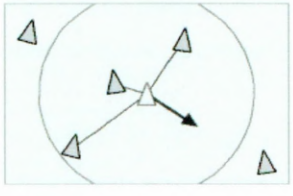


Figure 2. 3 Cohesion: steer to move toward the average position of local flock mates. This corresponds to attraction of the Boids to each other.

These behaviours are described mathematically in the model and the interaction between local, simple behaviours of individual Boids produces complex, organized global group behaviour (applet demo available on line at: http://www.red3d.com/cwr/boids/) Fig. 2.3.

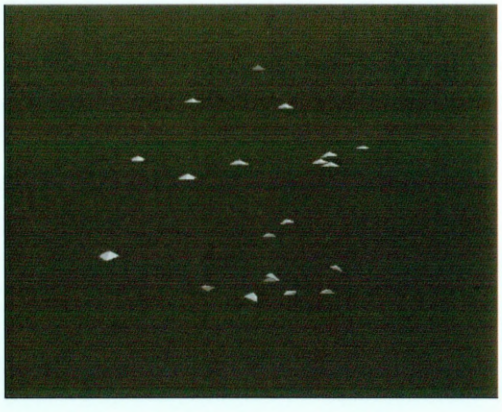
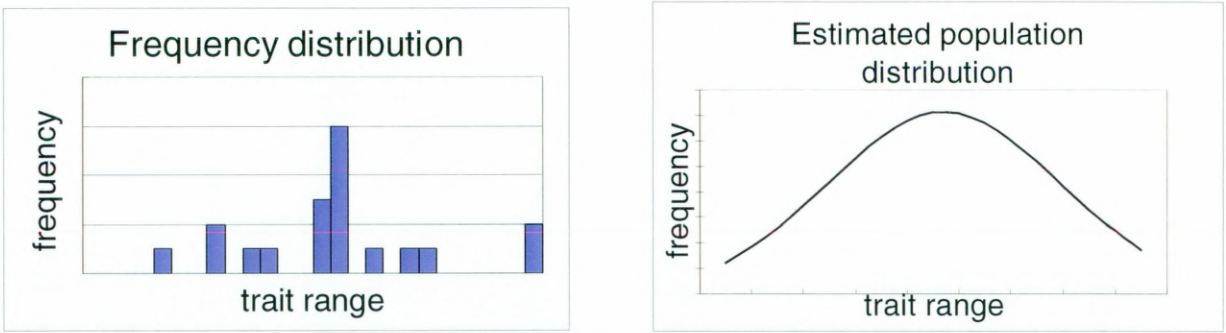


Figure 2. 4 Flocking of individual Boids as a result of the three steering behaviours. The Boids are represented in the diagram as pyramids.

An advantage of IBMs is that the trait values can be directly linked to experiments. For example, as in Pachepsky *et al.* (2001), experiments may be undertaken on a small number of individuals of a species to determine values for specific traits. This set of measures of individuals may then be

used to represent the species by fitting statistically a distribution to the observed range of measurements. Figure 2.5 a) shows the frequency distribution of values from a given trait and 2.5 b) the fitted distribution, taken from the plant community dynamics of Pachepsky *et al.* (2001). This process may be repeated for all traits and so modelled individuals may be constructed by sampling from the distribution of each trait. As a result IBMs can offer a valuable link between experiment and theory.



(a) (b)

Figure 2. 5 (a) Experimentally derived trait frequency distribution and the corresponding estimated population distribution for that trait from Pachepsky *et al.* (2001)

System level patterns, e.g. community structuring, can also be obtained from the field and laboratory to test the validity of the model results. For example, models based on bacterial colony formation have used the results of agar based colony experiments to validate their model hypotheses (Kreft *et al.* 2001).

IBMs may be considered a reductionist approach, as the system is decomposed into smaller elements, i.e. the individuals and, in most cases, the dynamics emerge from local rules/interactions only. However, if the system exhibits emergence, e.g. the flocking seen in Boids, then it can be argued that the macroscopic patterns observed are not reducible to the individuals of the system but to the individuals and their interactions. Consequently, the collective and emergent behaviour of systems may not be understood simply in terms of the characteristics of the individual parts but must take account of the couplings among those parts (Wolf and Holvoet 2004).

2.1.3 Cellular Automata

Cellular Automata (CA) are spatially explicit grids containing cells that are in one of a finite number of states (Wolfram 1986; Kazakov and Sweet 2000). An IBM may be considered a CA if it is spatially explicit, grid-based (cells contain discrete values) and the individuals are immobile (Reynolds 1995). They can be of one or more dimensions although commonly they are one or two-dimensional. Since the discrete grid cannot be infinite, and each cell must have the same number of neighbours, boundary conditions must be imposed. Common boundary conditions are reflecting and periodic, and are chosen to reflect the realism of the underlying problem. The periodic boundary conditions come closest to simulating an infinite lattice (Fig. 2.6), and reflective boundaries are used when the problem studied also has a boundary (Fig. 2.7).

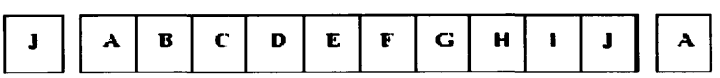


Figure 2. 6 Periodic boundaries in one dimension. The ten cells in the centre represent the lattice that is being updated, the two cells at the end are boundary cells that receive copies of the cells at the opposite end of the lattice at each time step.

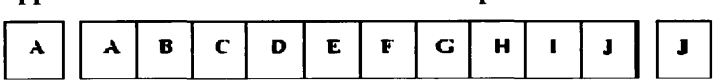


Figure 2. 7 Reflective boundaries in one dimension. The ten cells in the centre represent the lattice that is being updated, the two cells at the end are boundary cells that receive copies of the cell closest in the lattice i.e. reflection at each time step.

Time is also discrete and the state of a cell at time t is a function of the state of a finite number of cells, termed the neighbourhood, at time t-1 and a transition rule. Common neighbourhoods in 2-dimensions are the Von Neumann 4-cell neighbourhood and the Moore 8-cell neighbourhood Fig. 2.8.

(a) (b)

Figure 2. 8 a) the four cell Von Neuman neighbourhood, b) 8 cell Moore neighbourhood

The transition rule(s) is the updating mechanism, based on the values in the defined neighbourhood. All cells are updated simultaneously and each time the rules are applied to the whole grid a new generation is produced.

Very few CA and spatially explicit IBMs possess analytical solutions. Therefore most require numerical solutions, as the mathematical model governing the individual's behaviour may possess many parameters and nonlinear functions. Further, the incorporation of explicit space makes analytical methods elusive. Key questions when developing any spatio-temporal model include setting the correct spatial and temporal scale, deciding what individual-level state variables should be included and how to model the behaviour of that individual.

The Game of Life is the best-known Cellular Automata (Wolfram 2002) and its cells can be in one of two states, dead or alive. The transition rules are as follows:

- A living cell with 0 or 1 living neighbours dies from isolation;
- A living cell with four or more neighbours dies from overcrowding;
- A cell with three living neighbours becomes alive;
- all other cells are unchanged.

Despite the simplicity of the rules a diversity of complex behaviour is achieved based on the initial configurations of the universe, fluctuating in space and time between apparent randomness and order. Cellular automata are defined by local rules of interaction yet behaviour on a larger scale often occurs and this behaviour is said to be emergent. CA have been widely used in biological and ecological fields to study the spread of clonal organisms (Inghe 1989; Silvertown *et al.* 1992), drosophila dynamics (Dytham and Shorrocks 1992) and bacterial and fungal colony forms (Ben-Jacob *et al.* 1994; Halley *et al.* 1996; Ermentrout and Keshet 1993). CA also have practical value for solving diffusion problems (see below, section 2.2.1) in physical systems (Brown and Rothery 1983).

2.1.4 Emergence and Self-organisation

Potential characteristics of Cellular Automata and IBMs are emergence and self-organisation. Emergence arises as a global, complex macro-pattern from the interaction of micro-parts as defined by simple rules (Crutchfield 1994). It generates a novel global property that cannot be deduced from the characteristics of the individual parts alone. Emergence is typically robust in accordance with the interacting parts, i.e. the failure of one or a small number of interacting parts will not result in the collapse of the emerged global scale pattern. Emergence arises in many fields and the parts can be anything from neurons or individual organisms to components of a network and stars: the type and size of the parts depends on the field of study. The individual's immediate surroundings include other individuals in a domain-specific neighbourhood and the environment. Importantly, emergent behaviour is not predictable from the description of the fundamental components of the system, and no characteristic of the fundamental component would lead one to predict the global property. For example there is no characteristic of an individual bird that would lead one to predict a flocking property when many birds interact. Another example is the interaction of neurons leading to intelligence. Consequently, the emergent global property is not created by a global rule or algorithm. It is generated by the behaviours of the interacting parts.

Self-organisation is a dynamical and adaptive phenomenon where systems acquire and maintain new system structures without explicit pressure from outside the system environment (Wolf and Holvoet 2004). Self-organisation can be considered an increase in system behaviour that enables the system to acquire spatial, temporal or functional structure without external control, i.e. autonomously. Where an increase in dynamic order arises from external pressure this is not self-organisation. A self-organising system is expected to cope with perturbations and adapts to maintain its organisation. Finally self-organising systems are dynamical and so the system has to be far from equilibrium, reacting to the environment and, as a result, autonomously self-organising towards a certain attractor in state space, i.e. towards certain organised behaviour. Many biological systems exhibit self-organisation including: the organization of cyclic molecules to form regular nanotubes within a cell (Mason 1997); morphogenesis, the process of how a living organism develops and grows (Murray 1989); formation of bacterial colonies (Ben-Jacob *et al.* 2003) and the

formation of *Dictyostelium discoideum*, a slime mould that arises from self-organised aggregation of the unicellular amoebae as a response to starvation (Nicolis 1992).

Commonly, systems that exhibit self-organisation may also exhibit emergent properties, as both are dynamical systems changing over time, that are robust to perturbations. However, one can occur in the absence of the other. The properties that are specific for emergence, but not needed for self-organisation are novelty, micro-macro effect, flexibility with respect to entities and decentralised control. Multi-agent systems are autonomous and increase order through interactions. A self-organised system may not exhibit emergent properties, i.e. properties that are novel, when every agent has a description of the global behaviour to be achieved. An example of this may be the self-organisation of the human body: all human cells self-organise from stem cells to become more function-specific cells such as liver, heart, bone or lung cells. If the fate of the human stem cell is predetermined by design then the system is self-organising, but not emergent, as there exists a global rule governing the fate of the stem cell. Likewise emergence occurs without self-organisation, e.g. thermodynamics emerges from statistical mechanics in a stationary process and so non self-organising system (Crutchfield and Feldman 2001). A stationary process is one where there is no increase in order, and therefore no self-organisation. The boundaries between the concepts of Self-organisation and Emergence are blurred and the two terms are often conflated (Wolf and Holvoet 2005). There exists no definitive definition of either concept or formally proven quantitative indicators (Shalizi *et al.* 2004) to characterise the degree to which a system self-organises or exhibits emergence.

2.1.5 Process-based models

Process-based models incorporate a mathematical description of the biological and environmental processes during the lifecycle or, specific stage of the lifecycle, of the modelled components. Indeed most IBMs focus on the stages of the individuals life-cycle and are process based (Grimm and Railsback 2005), and so these processes include the characteristics of the individual that are to be tracked through time. The plant community dynamics model of Pachepsky *et al.* (2001) again

serves as a useful exemplar. The model is individual-based and characterises the physiological processes relating to the lifecycle and functioning of the plant including resource capture area, resource uptake, internal allocation of resource between structural, storage and reproductive stores, time to reproduction, number of progeny produced, dispersal of progeny and survival of the plant. Each of these processes is represented by an algorithm that is parameterised by traits. The combination of a functional, process-based representation of the system under study and individuals as the accounting unit of that system provides a useful platform for investigating the relation between individuals and community.

2.1.6 Linking individuals to communities

The previous work of Bown (2000) and Pachepsky *et al.* (2001) has led to a framework to link properties and processes at the scale of the individual to patterns at the scale of the community such as (functional) diversity. Representing thousands of uniquely defined individuals in an ecosystem introduces a conceptual challenge and Bown (2000) defines an abstraction to manage this complexity, trait space. The axes of trait space are the set of traits defining the individuals, and each individual occupies a point in that space. Diversity in the community is represented by the distribution of points in trait space. Importantly, this abstraction facilitates identification of associations among individuals that lead to community-scale phenomena. For example, Pachepsky *et al.* (2001) identified a trade off between two traits (time to fecundity and reproductive biomass) that promoted community-scale coexistence. This trade-off leads to a temporal separation of reproductive events for different functional types, therefore reducing competition for space and allowing coexistence of types.

2.2 Generic model processes

Clearly, the processes included in a functional representation of a system depend on the nature of that system. Here, our remit is ecology and typical lifecycle processes of individuals in ecosystem models include movement, growth, reproduction and death. These processes require explicit mathematical descriptions to be integrated within an IBM model. Existing IBMs have used random

events for death (Pachepsky *et al.* 2002). Reproduction is usually clonal (Pachepsky *et al.* 2001) and has been modelled using either lifecycle or random events, although some models are now considering the implications of gene flow (Takenaka 2001; Eberst *et al.* in preparation). Movement and growth have been commonly modelled as a transport mechanism using diffusion (Okubo 1980; Kreft *et al.* 2001), and this approach is pertinent here for the development in space of fungal colonies. Diffusion has also been used to represent spatial expansion and contraction and the spread of disease. The implementation of movement or growth using diffusion is complex and the diffusion concept is described below.

2.2.1 Diffusion

Diffusion models mechanisms of transport mathematically and describes the spatial pattern of abundance and concentration over time. Over longer time scales diffusion introduces non-local effects as the impact of local neighbourhood permeates through space.

Diffusion occurs from regions of high to low concentration and this flux is proportional to the concentration gradient (Brown and Rothery 1993). The one-dimensional diffusion equation for a concentration u is (Crank 1975):

$$\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2}$$

where D is the diffusion coefficient measured in units of distance^2 per unit time. The diffusion coefficient is a constant that governs the rate of diffusion.

Analytical and numerical methods exist for solving diffusion equations and the ease with which an analytical solution may be found is dependent on initial and boundary conditions as well as the complexity of the equation set that the diffusion is part of. Analytical solutions for a simple diffusion process may be obtained for a number of simplified initial and boundary conditions provided the

diffusion coefficient is constant. The one-dimensional analytical solution of the spread from a point source over an effectively infinite region in both directions has the form (Crank 1975):

$$U(x,t)\,u = \frac{M}{2(\pi Dt)^{\frac{1}{2}}} \exp\left(\frac{-x^2}{4Dt}\right)$$

This describes the spreading by diffusion from a point source of magnitude M deposited at time t = 0, x =0. Fig. 2.9 graphically depicts the distribution of concentration from the point source at x=0 at times t=1,2,4,8 and 16.

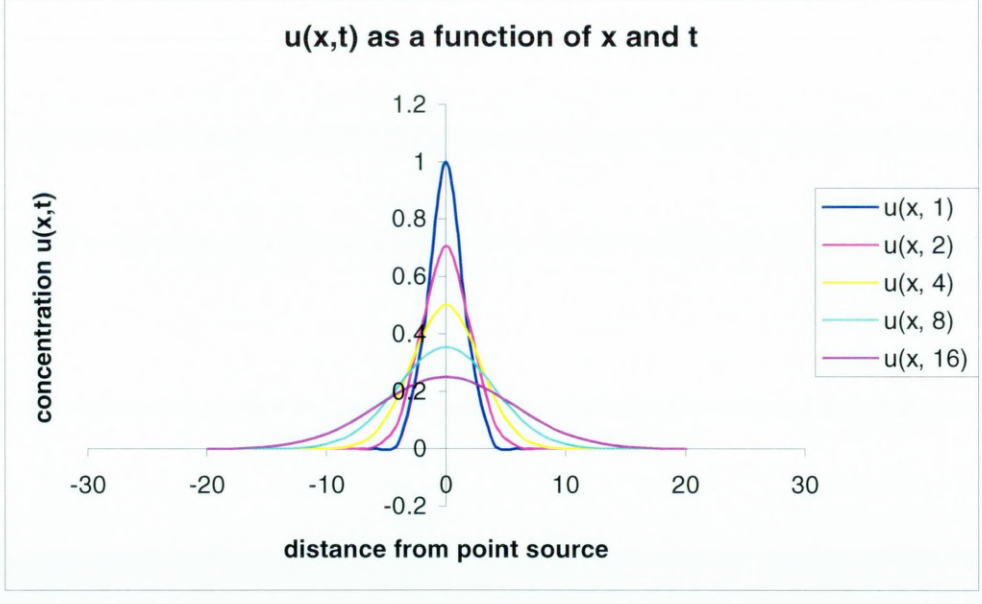


Figure 2. 9 Distributions of concentrations emanating from x = 0 at times 1, 2, 4, 8 and 16.

The above analytical solution can be verified by differentiation since this provides concentration values at the corresponding spatial and temporal points.

Realistic biological processes modelled via diffusion, i.e. those that describe real phenomena, are to be satisfied in complex domains (e.g. soil environment), have non-constant diffusion coefficients

and are subject to complex initial and boundary conditions. Analytical solutions in these cases are very rare and numerical methods are used to determine the solution (Crank 1975).

2.2.1.1 Numerical solutions to Diffusion equation

Euler introduced the finite difference scheme (Atkinson 1978) that is now used to determine the numerical approximation of partial and differential equations (details in Appendix C). This is based on replacing the differential terms with difference terms. The FTCS (forward time centred space) finite difference scheme can be used to solve a simple diffusion process, i.e. no nonlinear reaction terms, a constant Diffusion coefficient and one that is not coupled to any other Partial Differential or Differential Equations.

The finite difference explicit method results in a stability criterion that is dependent on the grid resolution ratio in space and over time. Recall from section 2.1 that a numerical method discretizes the domain: k and h refer to the temporal and the spatial discretization respectively. The grid size ratio must obey the condition in Crank 1975:

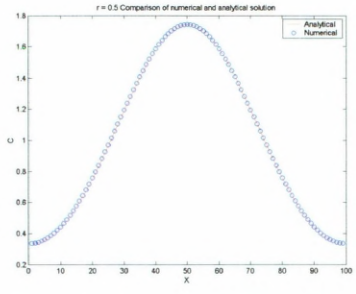
$$r = \frac{kD}{h^2} <= \frac{1}{2}$$

The value of r is critical and if it is increased above 0.5 (denominator on the RHS relates to the number of neighbours in the neighbourhood, 2 for a 1D system) then the solution will bear no resemblance to the analytical solution, becoming unstable and errors increase without limit. The physical interpretation of this is that the maximum allowed time step is the time for diffusion across a cell of width h (Press 1992). This stability condition forces a large number of small time steps (k) to be taken. Figure 2.10 a and b demonstrate the numerical solution of a 1D system with r values obeying and not obeying the stability criterion (respectively) and with initial conditions equal to:

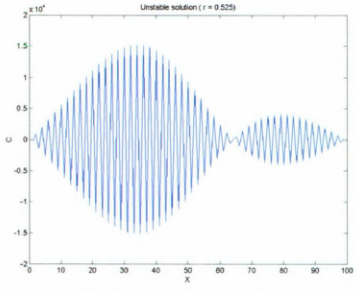
$$u(x, j) = \exp\left(\frac{-(x-\mu)^2 / 2\sigma^2}{\sqrt{2\pi\sigma^2}}\right).$$

This corresponds to an initial concentration in the shape of a parabola rather than a point source as in 2.2.1.1 and is a commonly modelled system. Boundary conditions are imposed, as in reality the domain is not infinite. The boundaries are reflecting implying that there is no flux of particles either into or out of the domain (Grindrod 1991), i.e. it is a closed system.

Figure 2. 10 Effect of r on the numerical solution



(a) r =0.5 is a good approximation

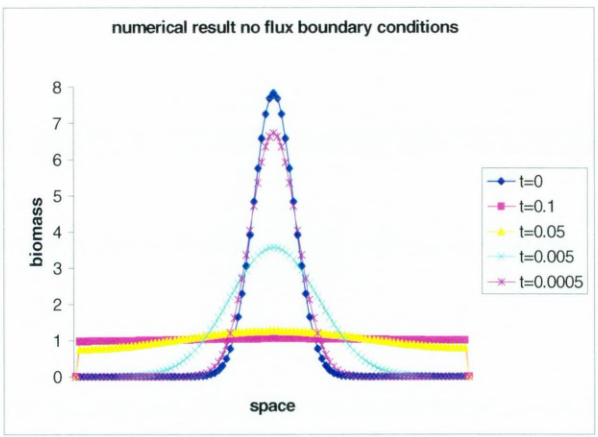


(b) r =0.525 is a poor approximation

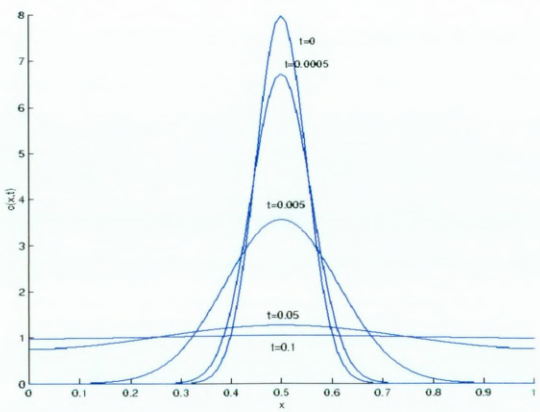
The values of k and h are not arbitrary and are informed by the physical system being modelled, the specification of the computer used to solve the diffusion process and the diffusion coefficient of the mobile phase/chemical. The physical length of the system is the product of h and the number of grid points used in the simulation; similarly the real physical time a simulation corresponds to is the product of k and the number of time steps. If the spatial and temporal boundaries are known for the physical system then appropriate k, h and D vales can be determined based on the maximum size of grid the computer can process. Since the number of grid points (size of grid) and time steps used in a simulation is constrained by the specification of the machine being used there exists a trade-off between accuracy (small k and h values, large number of time steps and grid points) of the solution and the speed in which a solution is generated. For example the experimental system is a Petri dish that is approximately 5cm in diameter and a fungal colony is inoculated in the centre. If the extension rate of a typical colony is 0.00585 m/h it will take the colony approximately 3.41 hours to grow 0.02m. If the system is to be modelled using 128 grid points then the value of h is 0.02/128 = 0.00016. If there are to be 15 time steps the corresponding k value is 0.533. Noting the stability criterion and setting r = 0.5 then the corresponding diffusion coefficient is $1.07 * 10^{-7}$ h/m^2.

2.2.1.2 Validation of explicit numerical algorithm

In order to test the robustness of an implementation of a numerical method it is good practice to test it against the corresponding analytical result, since one can test the underlying numerical methodology by simplifying the equation used. The numerical results should be a good approximation of the analytical results. Here, the one-dimensional implementation of the numerical diffusive process, to be used in the original version of the model (prototype is detailed in Appendix B), was implemented in C ++ (Visual Studio 6) and tested against the known analytical solution with initial and boundary conditions equal to that in 2.2.1.1. As the analytical and numerical solutions are significantly similar ($p < 0.05$) this verifies the reliability of the implementation of the underlying algorithm. The analytical result of the diffusive process was consistent with the numerical result in one-dimension. (Fig. 2.11a) and b)).



(a)



(b)

Figure 2. 11 Comparison of numerical (a) and analytical (b) result of 1D diffusion process

2.2.1.3 Shortcomings of explicit algorithm

The FTCS finite scheme is limited to simpler problems. More complex problems with nonlinear reaction terms that result in rapid changes in the solution and non-constant diffusion coefficients require extremely small values of k, otherwise a good approximation is not achieved. Results using small values of k are sometimes not achievable computationally and therefore limit the application of the explicit algorithm. A numerical scheme that allows larger time steps whilst maintaining accuracy is the Crank Nicholson method (Ortega and Poole 1981).

2.2.1.4 Crank Nicholson method

The Crank Nicholson (CN) scheme is referred to as an implicit scheme as it uses part of the current solution and part of an implicit future solution, i.e. at time, t+1, to extend the stability condition. It is therefore an average of a fully explicit and implicit method. In an explicit method the solution can be calculated from known values at the current time step. However, an implicit method's formula is based on unknown values at a future time step. Whenever there exists a number of unknowns, a system of simultaneous equations can be solved to determine the corresponding unknown values. The CN method increases the numerical stability at the expense of having to solve a set of linear (simultaneous) equations to obtain the 'future' solution. In terms of diffusion the number of unknowns will be based on the resolution of the grid and must be solved in order to progress to the next time step. Common methods for solving a vast number of simultaneous equations include direct methods, e.g. Gaussian elimination, and iterative methods, e.g. Gauss- Seidel method. Iterative methods will generally be more effective than direct methods for solving equations in three dimensions as they are faster. However for nonlinear equations iterative methods are a necessity (Ortega and Poole 1981). The Crank Nicholson method does not impose any restrictions on the spatial and temporal intervals but the larger the temporal resolution the less accurate the solution. The accuracy of a method is determined by the components of the truncation error as discussed in Appendix C. In the Crank Nicholson method the truncation error includes terms of the second and higher order, i.e. these terms are small, and so the method is considered second order accurate. The fully implicit and explicit method are 1^{st} order accurate with respect to time and 2^{nd} order accurate with respect to space since the error includes first order terms associated with time and

space respectively. The explicit method also possesses a stability criterion. The CN method therefore is the best of both worlds in terms of accuracy and stability. Further detail of the Crank Nicholson method and the testing of its implementation in this thesis is outlined in Appendix D.

Chapter 3. The development of the conceptual model

3.1 Role of modelling

Modelling is not a replacement for experimentation and if a model's role is to obtain an understanding of the desired system then it must be linked to experimentation. Indeed, if an understanding could be achieved from experiments alone, then it would be unnecessary to develop a model. When experimentation is not practical, e.g. for soil systems in which the science of soils – mapping the matrix of plant, animal, insect and microbial communities in a habitat - is in its infancy and prohibitively difficult, then modelling may be utilised to bridge the gaps between simplified experiments and the natural system. Modelling is also used as an aid to intuition where system complexity makes the interpretation of experimental data overwhelmingly difficult. The main goal of this thesis is to use modelling to determine the key processes responsible for the mycelial distributions of individual and interacting fungi. The model will link genotype and environment to the manifest phenotype of the fungal colony. If a model is to be used to gain insight into the processes responsible for fungal community dynamics in a natural environment then it must:

1. Be linked to experimentation since this affords effective model parameterisation and validation
2. Represent space explicitly, as the heterogeneous environment plays a fundamental role in shaping observed mycelial distributions
3. Encapsulate the key fungal processes associated with growth and development: namely uptake, transport, recycling and growth
4. Define individuals in terms of physiological traits that link growth and form to functional consequences

5. Reflect the complexity of fungi in terms of their self organisation and responsiveness to their environment
6. Account for local and non-local effects of colony growth
7. Extend to model interactions where each individual colony can have an explicit representation in terms of traits

3.2 Existing theoretical models of fungal growth

The earliest models of colony growth focus on the hyphal scale and are designed to elucidate the mechanisms of hyphal tip growth, branching and anastomosis (Bull and Trinci 1977; Prosser and Trinci 1979). These models were either mean-field or Cellular Automata based. The disadvantage of these models, however, is that they do not consider the impact of non-local effects on the growth, branching and anastomosis of hyphal tips. In addition, they do not address how the cooperative, collective behaviour of fungi affect colony-scale macroscopic growth. Furthermore, it is unlikely that these models could be extended to deal with the many hyphal interactions among different colonies.

Davidson *et al.* (1996) present a colony-scale model based on a diffusible, replenishing substrate, an agent converting substrate into energy that drives the proliferation of biomass, and diffusion of an activator. Davidson *et al.* (1996) show that by varying key parameters many of the observed morphological patterns produced by growing fungal colonies may be obtained. Although phenomological, the model illustrates the important point that relatively few processes can be orchestrated by different contexts to produce wide-ranging phenotypes, and this capacity must be embodied in any general representation. In this modelling framework each individual colony lacks an explicit representation and no physiological interpretation of the process responsible for complex structures was attained.

More recent theoretical approaches couple two, or more, characteristic scales in the hope of understanding what drives colony growth dynamics. These models have evolved from early work by Edelstein (1982) and Edelstein and Segel (1983), describing colony-scale growth in terms of

hyphal density and density of hyphal tips, accounting for different hyphal mechanisms such as lateral and dichotomous branching, anastomosis and translocation of metabolites within the mycelium. Davidson and Olsson (2000) and Boswell *et al.* (2003) developed this work further by producing a model based on directed growth of fungal hyphae, and include more realistic, bi-directional translocation mechanisms. Boswell *et al.* (2002) identify vacuolation as an important process. However the 'inactive hyphae', i.e. those hyphae not involved with translocation, are degraded into the environment and therefore not recycled by the colony. These models have been useful in investigating the interplay between active and passive translocation within the mycelium for homogeneous and heterogeneous environments, and suggest that passive translocation is used for random exploration while active translocation is used for resource exploitation, (Boswell *et al.* 2002). Stacey *et al.* (2001) also extend the work of Edelstein (1982) to address transmission rates of fungal soil-borne pathogens from infected to susceptible plant. Although offering valuable insights into the questions these models are designed to address, all of the models described above lack an explicit account of an individual, a biomass recycling mechanism and the modelling framework is 2-dimensional. The only known 3 dimensional fungal growth model is that developed by Meskauskas *et al.* (2004). This is a vectorial-based model but this also lacks a biomass recycling process and the emerged colony structures are a consequence of local processes only.

There exist fewer community scale fungal models. Halley *et al.* (1996) and Bown *et al.* (1999) adopt a similar Cellular Automata approach although the fundamental assumptions of the models are opposing. In Halley *et al.* (1996) the cooperativeness of fungal colonies is ignored, and so the colony is treated as additive assemblages and the emerged behaviour of the colony is a result of local rules only. Halley *et al.* (1996) recognise this and state "by regarding the mycelium as a simple consumer of resources we have ignored its more "intelligent" properties (Rayner 1988)". This model was developed to illustrate the potential interest and utility of a CA approach rather than to capture the full complexity of mycelia (Halley *et al.* 1996). The developed CA model by Bown *et al.* (1999) examines the relative contribution of local and non-local effects on the dynamics of a two-species fungal microcosm. This model represents implicitly the physiological processes that

are important in the outcome of individual fungal interactions. The analysis shows that the outcome of interactions at the local scale depends on the larger scale context, and so the behaviour that emerges cannot be understood in terms of the behaviour of isolated modules, i.e. local behaviour only, highlighting the need to link across scales. These models were useful in the particular investigations that they were developed to be manipulated in, but again, neither have an explicit representation of an individual in terms of trait values that govern explicit physiological process or incorporate a recycling element. Given the modelling criteria listed in 3.1 and the limitations of existing theoretical models it appears that aspects of both IBM and CA could be exploited to model the development of individual fungal colonies and, later, fungal community dynamics.

3.3 The way forward

No existing model of colony growth meets all of the criteria of 3.1 above. The model presented here is a hybridisation of features from the IBM and CA paradigms since space has to be explicitly represented (Criterion 2). The model will be investigative in nature, as an understanding of the link between the description of colony behaviour, and the emergent mycelial distributions is important. By adopting an Individual Based Modelling approach each fungal colony can have an explicit representation in terms of traits representing the individual's genotype (Criteria 4, 7). Further, by making the model process-based these traits will result from a mathematical description of the physiological processes relevant to the fungal life cycle or a particular aspect of it (Criterion 3). The nature of process based IBM modelling also supports the criterion of using real data to parameterise and validate the mode (Criterion 1). The trait values used to define the fungal colony should, in principle, be determined from experiments since process-based models are amenable to experimental parameterisation. Observed colony forms and interaction outcome patterns can be used to validate the model for the colony and community scale respectively.

Since colony and community development is partly determined by local and non-local effects the developed process-based IBM must incorporate non-local interactions (Criterion 6). Diffusion is an appropriate process to incorporate into the theoretical model as diffusion is one of the simplest mechanisms to mediate both local and non-local effects. This can be achieved by incorporating

diffusion into at least one of the physiological processes of the model i.e. the contraction and expansion of the biomass or internal transport within the colony. Since fungal systems display characteristics of complex systems, any mathematical descriptions of the physiological processes will consist of partial or differential equations. These equations will contain many terms including nonlinear and the afore mentioned diffusive terms. Further, the environment in which the modelling will take place will impose non-trivial initial and boundary conditions, together with the possibility of a non-constant diffusion coefficient. Taking these factors into consideration a Crank Nicholson method, or another implicit method, is required to numerically solve the equation set.

The fungal community model will be derived from the single colony model and therefore the single colony model must be extensible (Criterion 7). If the extension to multiple colonies is to be practical computationally the complexity of the single colony model must be limited, admitting only the key processes. The extension will require the incorporation of fungal interaction outcomes that require more processes and parameters to be represented in the model.

The complexity of fungi can be reflected by IBM-based models as it is known that these systems produce complex behaviour from simple rules (Kreft *et al.*1998). The values of traits can be investigated to determine which individual trait(s) lead to specific instances of colony morphologies and coexistence of species. An investigation into the origin of this complexity in terms of heterogeneous mycelial distributions and species coexistence can thus be achieved by determining the nature of these simple rules that reflect the fungal life cycle (Criterion 5).

We present a modelling framework that adheres to the Criteria of 3.1 drawing from the modelling paradigms discussed in this Chapter 2. The remainder of this chapter describes the mathematical formulism of the simple rules of the model, based on traits that reflect the lifecycle of a fungal individual and which are experimentally measurable in principle.

3.4 Overview

Each individual colony is defined by a set of process-based behavioural traits that describe the impact of environment on behaviour. These traits correspond to parameters of an equation set that describes the growth and development of a fungal individual. An individual fungal colony is thus expressed as a collection of traits that define the manner in which it interacts with its environment via resource utilisation and growth.

In each iteration of the model the individual will uptake resource, recycle mobile and immobile biomass, redistribute mobile biomass, and grow according to the traits that govern these processes. Fig. 3.1 shows the interrelationships among the processes.

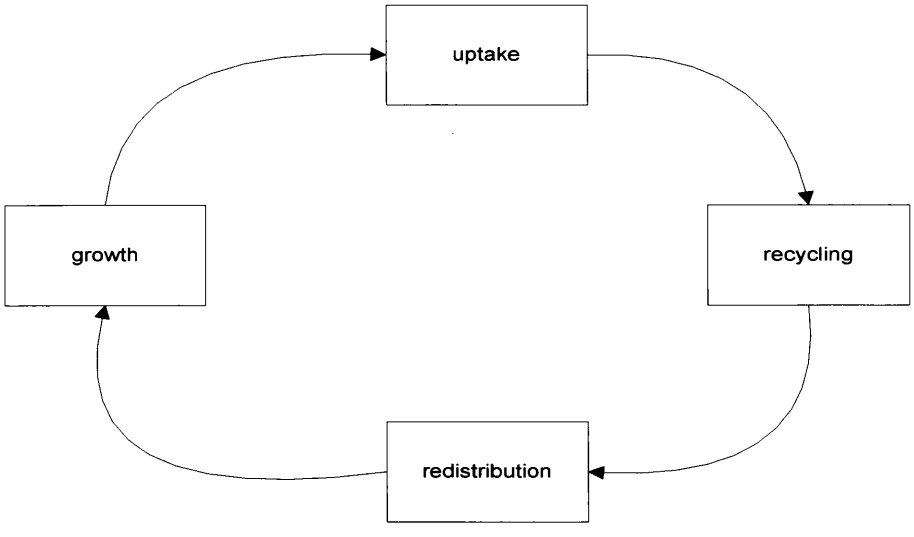


Figure 3. 1 Key processes of the model

The colony requires resource from the environment to persist and the colony growth pattern is dependent on the structural and resource distribution of the underlying environment. It acquires resource from the environment via uptake of nutrients; this uptake is converted into mobile biomass that may be transported within and utilised by the hyphal biomass of the fungal colony. An inter-

conversion process allows mobile biomass to be converted into, immobile hyphal biomass, and hyphal biomass may also be converted into the mobile phase. This interconversion enables the colony to recycle its biomass. Hyphal biomass is partitioned into two types; non-insulated that reflects active parts of the colony such as hyphal tips and insulated that represents older parts of the colony. Both types of biomass can interconvert between mobile and immobile biomass with prescribed rates. Finally, growth allows the colony to extend spatially in its environment, constrained by the physical structure of the environment. The colony is therefore represented as a mycelial network comprising of three types: immobilized insulated biomass, immobilized non-insulated biomass and mobilized biomass where these represent the biomass per unit area. The relative proportions of these components are dynamic and are determined by four key physiological processes.

3.5 Process Descriptions

3.5.1 Uptake

Non-insulated biomass is assumed to represent hyphae capable of significant uptake of external resource and corresponds mainly to active hyphal tips within a colony. Behind the tips, the cell wall and membrane change in character, and uptake is greatly reduced or absent (Carlile 1995) and is, in this sense, insulated. Common macroscopic manifestations of insulation include the formation of fully anastomised centres of colonies in nutritionally-depleted environments, and the formation of cord-like structures by some species in hostile environments (Rayner *et al.* 1999).Consequently, in the model, to allow for local communication between the internal and external environment both types of biomass, insulated and non-insulated, uptake resource from the environment (Fig. 3.2). Figure 3.2 demonstrates the computational stages associated with the uptake process. In all schematics, the boxes, ovals and parallel lines correspond to processes, traits and data stores respectively.

The uptake process is described by:

$$u = (\lambda_1 b_n + \lambda_2 b_i) r_e$$

where λ_1 and λ_2 are the uptake traits for non-insulated and insulated biomass respectively, b_n and b_i are non insulated and insulated biomass concentrations and r_e is the amount of external resource.

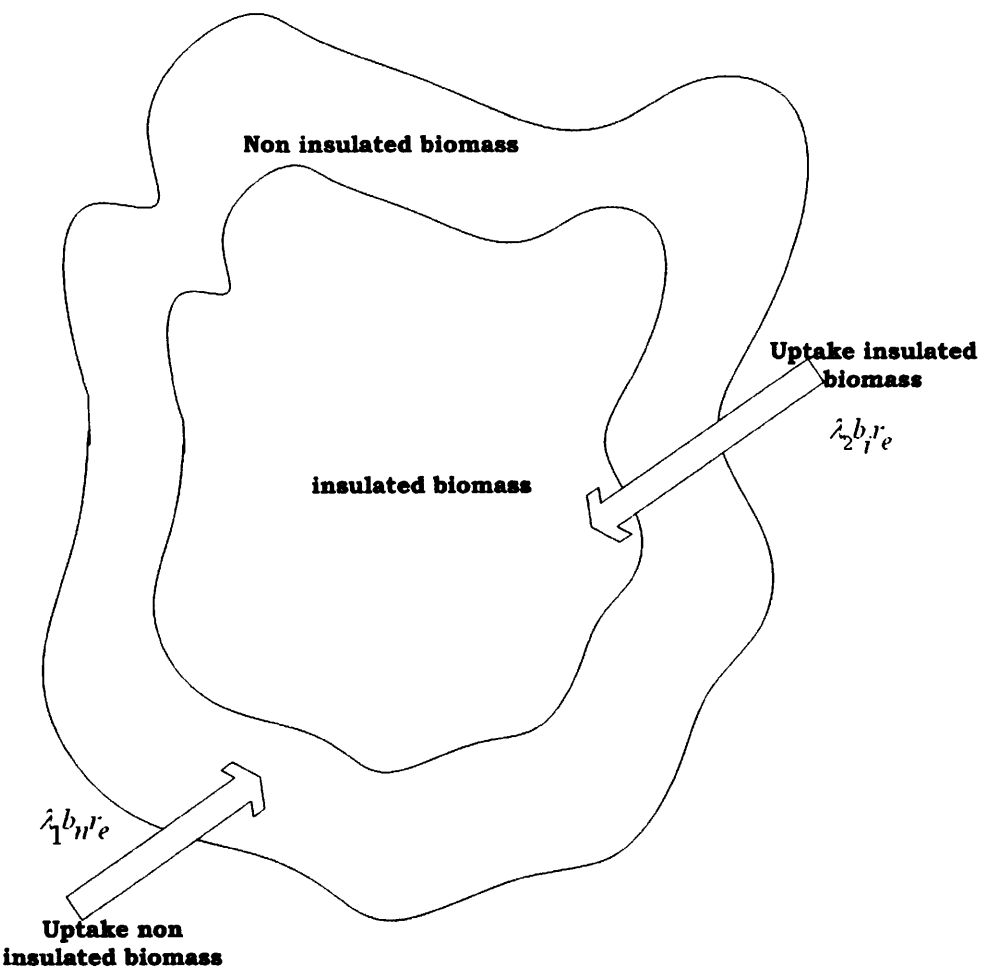


Figure 3. 2 Representation of a fungal colony and the uptake processes; the centre of the colony has become insulated and the outer edge is non-insulated biomass

The specific rate of uptake for non-insulated biomass, λ_1, is assumed to be larger than that for insulated biomass, λ_2, since it is well known that behind the hyphal tip, hyphae become rigidified and uptake is reduced significantly (Deacon 2005). We have assumed that there is a small uptake of substrate by parts of the mycelium represented by insulated biomass. This is used as a

surrogate for the underlying mechanisms responsible for the initiation of active uptake or new hyphal tips from insulated biomass in response to availability of new resources (see below). The resource taken up from the environment is converted with some efficiency into mobile biomass that is produced at a rate proportional to local uptake.

3.2.1.1 Uptake process definition

For each cell containing biomass:

1. If a cell contains insulated biomass calculate the amount of uptake given the insulated uptake trait value (λ_2), the amount of external resource (r_e) and biomass (b_i).

2. If a cell contains non-insulated biomass calculate the amount of uptake given the non-insulted uptake trait value (λ_1), the amount of external resource (r_e) and biomass (b_n).

3. If the total calculated uptake (insulated + non insulated) is greater than the amount available then assign the total site/cell acquisition equal to the available external resource.

4. If the total calculated uptake (insulated + non-insulated) is less than or equal to the amount available then assign the total site/cell acquisition equal to the total calculated uptake.

3.2.1.2 Uptake Schematic

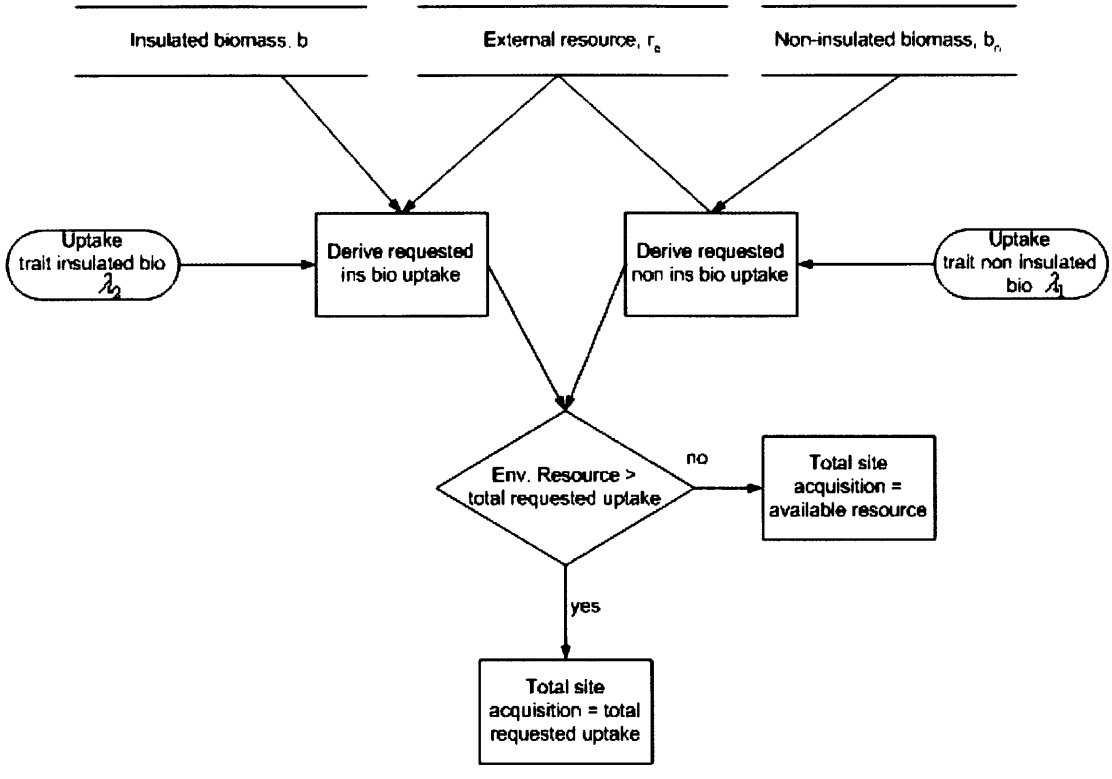


Figure 3. 3 depicts the computational steps associated with uptake of resource from the environment for each cell

The uptake trait value encapsulates the quality and quantity of resource and therefore reflects the effective uptake of the colony. Boddy *et al.* (1998) show that the quality and type of resource affects mycelial distribution. Once the nutrients are taken up by the colony they are converted into mobile biomass that may be converted into hyphal biomass, enzymes or reproductive structures. The model formulation also reflects via the linear relationship that primary resource capture is maximized by an increase in biomass and external resource (Fig. 3.4).

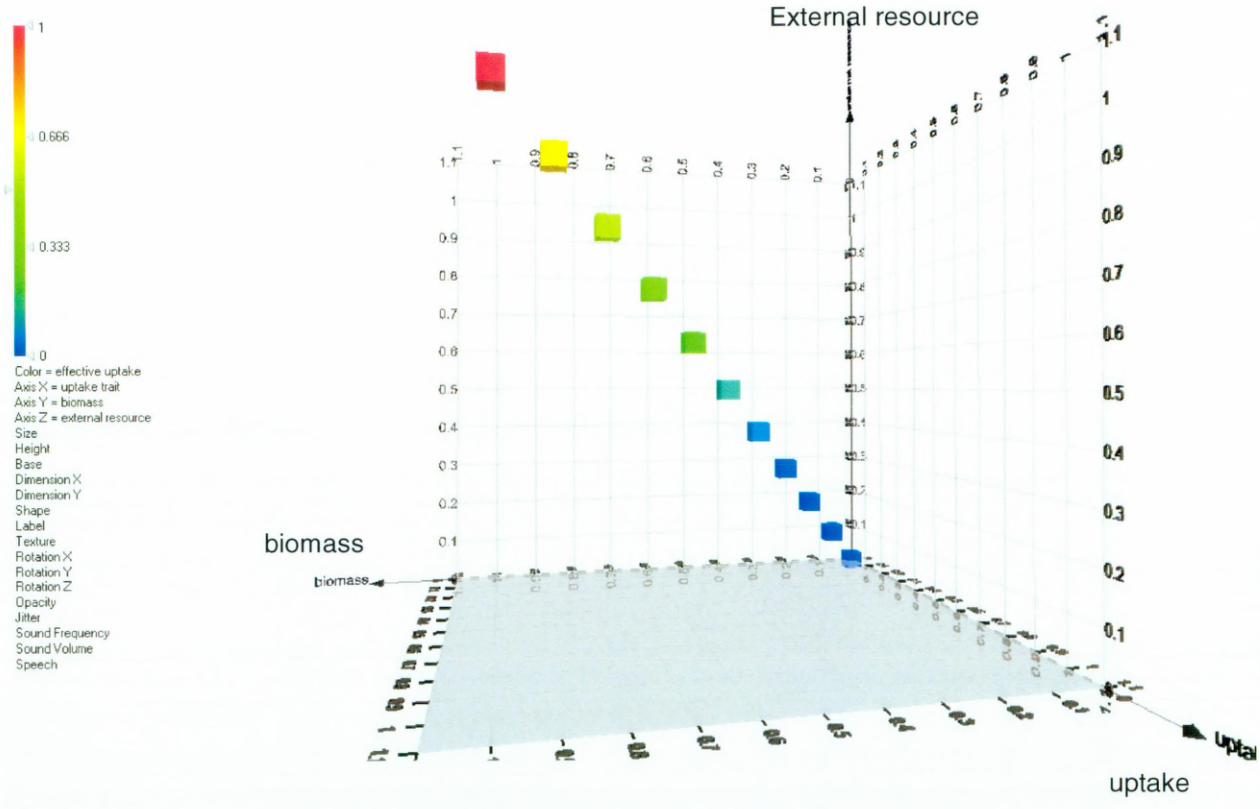


Figure 3. 4 Plot demonstrating the relationship between uptake trait (x axis), biomass (y axis) and external resource (z axis) on effective uptake rate (colour). An increase in one of these factors results in an increase in effective uptake.

3.5.2 Recycling

Immobile insulated and non-insulated biomass may be recycled, i.e. converted into mobile elements, and added to the mobile biomass pool. These remobilised elements are transported to growing tips via the movement of the internal mobile biomass component with concentration denoted by *n*. At this stage, we consider only that component of mobile biomass that is implicated in local biomass production. An explicit account of the various forms of mobile biomass (*e.g.* vesicles) and mechanisms for their movement, while possible in principle, would introduce excessive complexity that would obstruct our initial aim of determining the key processes. The key movement mechanisms include passive diffusion, active transport, pressure driven bulk flow, vacuolar compartmentalisation and vesicle trafficking (Olsson 1999) as discussed in the Chapter 1. These are encapsulated in the model by a single process governing reallocation of immobile

biomass. The precise mechanisms of transport and aggregation of mobile biomass, and in particular vesicles in hyphae, are not fully understood (Read and Hickey 2001).

Immobile insulated and non-insulated biomass are mobilised locally at prescribed rates, and the mobilisation process is assumed to consume elements of the mobile biomass.

We therefore set the mobilisation rate to be proportional to the local mobile biomass concentration in the hyphae (i.e. the ratio of mobile to immobile biomass)

$$\beta\pi, \text{ where } \pi = \left[\frac{n}{b_n + b_i}\right],$$

such that if the ratio is lower (higher) the rate of mobilisation per unit biomass will be proportionately lower (higher) as a direct result of a dilution (concentration) effect.

We assume that the coefficient for the rate of mobilisation β takes the value of parameters β_n and β_i for regions comprising non-insulated biomass and insulated biomass respectively.

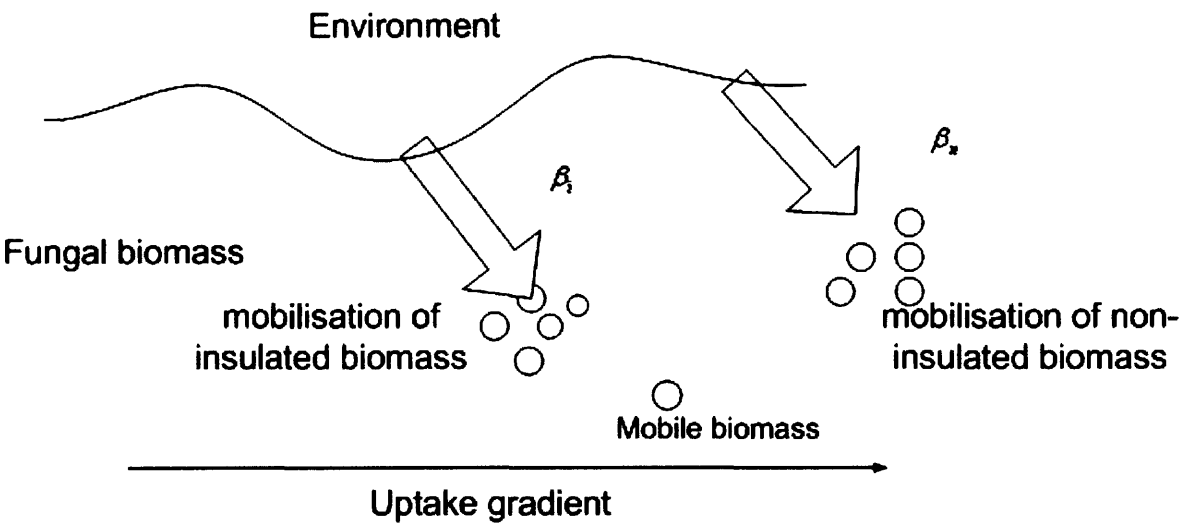


Figure 3. 5 Diagram representing mobilization, the rate of which is dependent on the local concentration of mobile biomass. With a low mobilization and no resource replenishment the mobile biomass gradient is positively correlated with the uptake gradient. The insulated biomass is negatively correlated with the uptake gradient.

Note that if the mobile biomass concentration is zero, the mobilisation rate is zero. Such behaviour is consistent with a mechanism for mobilisation involving assisted transport of elements of the mobile biomass across a membrane, *e.g.* active transport.

Mobile biomass may be used to build biomass and is assumed to be locally immobilised at a rate per unit biomass given by $\alpha\pi^{\theta}$ where the coefficient for the rate of immobilisation α assumes the value of parameters α_n and α_i in regions comprising non-insulated and insulated biomass respectively, and θ may be a constant.

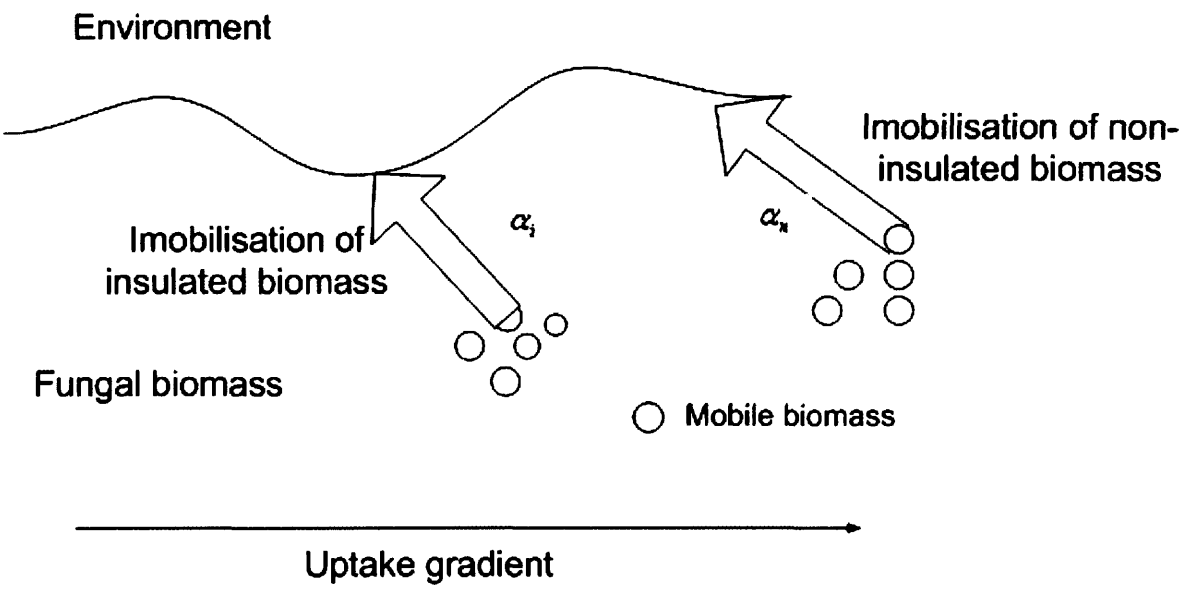


Figure 3. 6 Diagram representing immobilization. The rate is dependent on the local mobile biomass concentration.

To determine the local threshold value of π, which causes a switch from net mobilization to net immobilization, we set mobilisation equal to immobilisation and rearrange for π:

$$\alpha\pi^{\theta} = \beta\pi$$

$$\pi^{\theta} = \frac{\beta\pi}{\alpha}$$

$$\frac{\pi^{\theta}}{\pi} = \frac{\beta}{\alpha}$$

$$\pi^{\theta-1} = \frac{\beta}{\alpha}$$

$$\pi = \left(\frac{\beta}{\alpha}\right)^{\frac{1}{\theta-1}}$$

Thus for the nonlinear term, $\theta > 1$, then for sufficiently low values of π (i.e. $\pi < (\beta/\alpha)^{1/(\theta-1)}$) there is a net mobilisation of biomass, and this switches to a net immobilisation at higher values of π.

Mobile biomass is converted into immobile biomass with efficiency denoted by γ to account for metabolic cost of inter-conversion and the possibility that not all mobile biomass is allocated to immobile biomass production i.e. mobile biomass can also be converted into extra cellular enzymes and reproductive structures.

3.5.2.1 Recycling definition

Each cell containing insulated or non-insulated biomass is processed according to the recycling schematic below to obtain the mobile biomass distribution after recycling.

From the amount of mobile biomass derived from uptake the amount that will be immobilized, i.e. used to build insulated and non-insulated biomass, based on the trait values for the immobilization of insulated (α_i) and non-insulated (α_n) biomass rates, conversion efficiency (γ) and nonlinear term (θ) is determined. The amount of immobile biomass that will be mobilized i.e. converted from insulated and/or non-insulated biomass to mobile biomass given the trait values for the mobilization of insulated (β_i) and non-insulated biomass (β_n) rates and conversion efficiency (γ) is also evaluated. The flow diagram (Fig. 3.7) depicts the steps in the recycling process.

3.5.2.2 Recycling Schematic

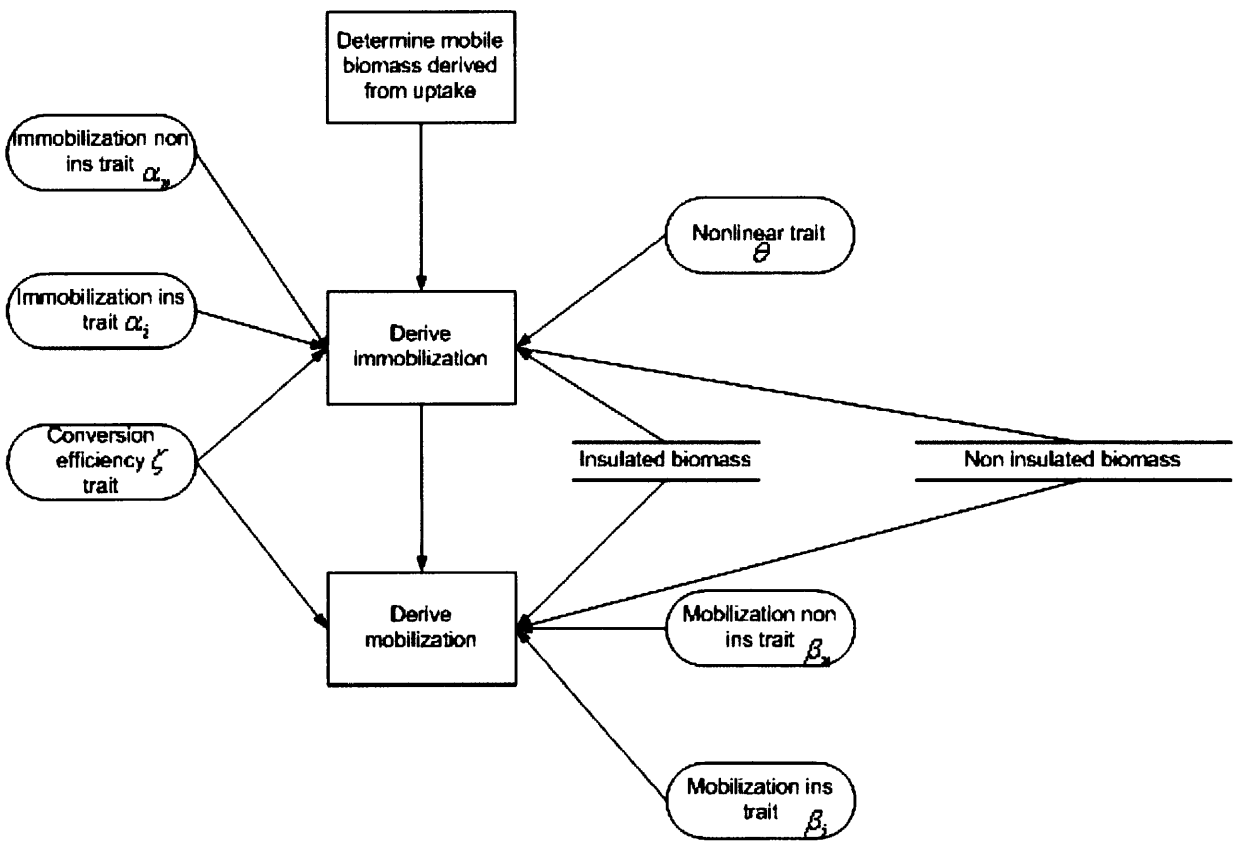


Figure 3. 7 The computational processes that describes the recycling process

Generally the recycling and transport of biomass allows the colony to reallocate older biomass to regions of the colony that require investment either in terms of exploiting fresh resource, warding off competitors or producing reproductive structures. Here, this is effected via different rates for the mobilization and immobilization of biomass for the insulated and non-insulated biomass (Fig. 3.1). For example if insulated biomass has a high mobilization and a low immobilization rate then older, aged biomass will be converted into mobile biomass that may then be transported and reused within the colony. If the non-insulated biomass has a high immobilization rate and a low mobilization rate this means the hyphal tips will adsorb mobile biomass promoting further fungal growth. This transport is governed by the redistribution process as described in 3.5.3. Different colonies may possess different capacities to recycle based on the trait values that control these processes. Fig. 3.11 illustrates the recycling processes undertaken by the two different types of

biomass together with the redistribution process, the redistribution process depends on the result of the recycling processes.

3.5.3 Redistribution

The integrated uptake of the mycelium together with remobilized components is transported within the colony, and this transport is controlled by the redistribution process and represented by a passive diffusion equation. Parts of the colony may have a greater local concentration of mobile biomass in areas where uptake and/or mobilization rates are high. If the diffusive process has a constant diffusion coefficient governing the rate of transport any peaks (hotspots) in the mobile biomass field (blue trend line) will be smoothed out eventually leading to a uniform concentration field in the steady state (pink trend line). The length of time taken to reach the steady state (no more change with respect to time) will depend on the diffusion coefficient and the concentration gradient.

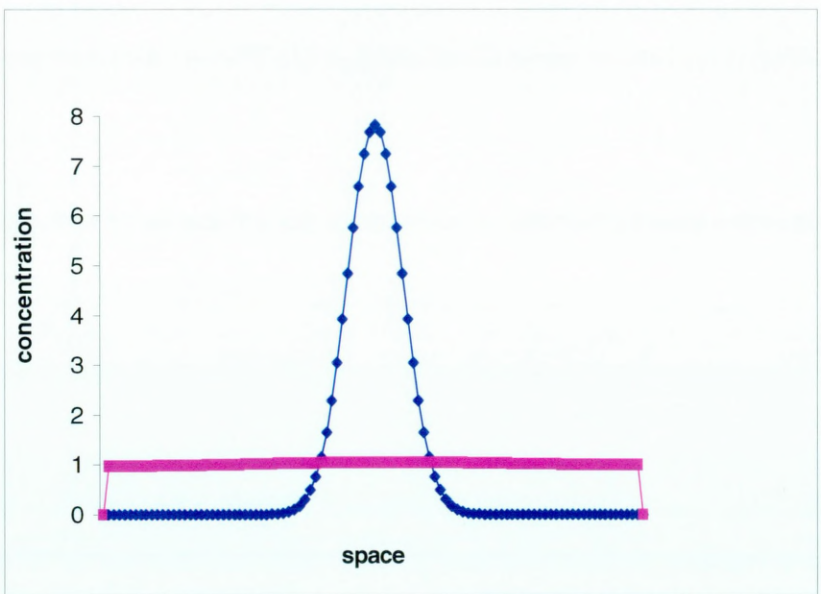


Figure 3. 8 The effect of a constant high diffusion coefficient, blue trend line represents the initial 1D concentration and the pink trend line the 1D concentration after some time.

If the diffusive process possesses a non-constant diffusion coefficient any local peaks in the field may be exaggerated or reduced depending on the value of the non-constant diffusion coefficient. If

areas of high concentration are inhibited from diffusing and regions of low concentration are promoted to diffuse then any local peaks will get larger with time (Fig. 3.9). The blue trend line shows the initial 1D distribution of mobile biomass concentration and the purple line shows the distribution at a future time step based on a non-constant diffusion coefficient that accentuates local peaks in a field.

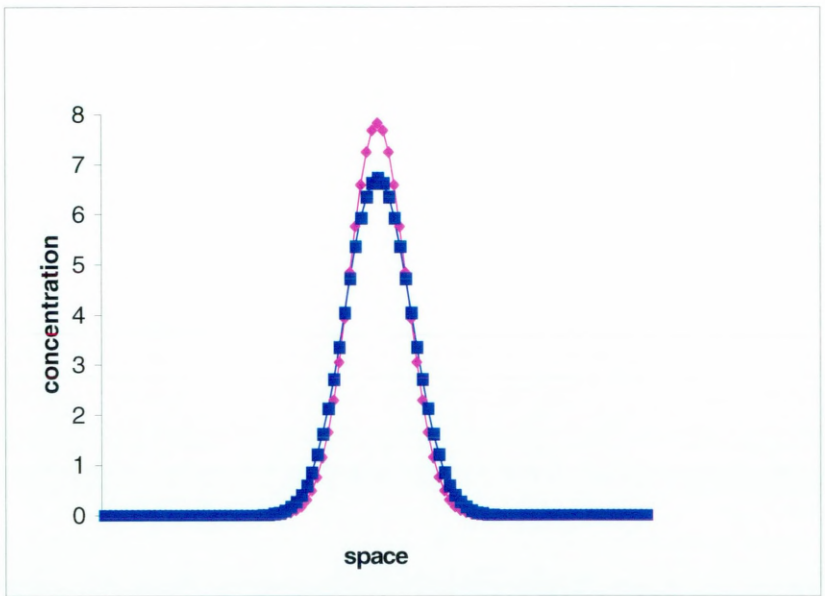


Figure 3. 9 Effect of a non-constant diffusion coefficient that may inhibits spread from peaks, blue trend line represents the initial 1D concentration and the pink trend line the 1D concentration after some time.

The diffusion of mobile biomass is described mathematically in two dimensions by:

$$\frac{\partial n}{\partial t} = D_n \frac{\partial^2 n}{\partial x^2} + D_n \frac{\partial^2 n}{\partial y^2}$$

where n represents the concentration of mobile biomass

x and y are the variables representing two dimensional space

D_n is the diffusion coefficient

The diffusion coefficient controls the rate of movement of mobile biomass. For the transport of the mobile biomass the diffusion coefficient is non-constant, and so this transports mobile biomass in

the direction of regions utilising fresh hotspots of resource, promoting further exploitation. If the mobile biomass concentration is above a threshold value then the diffusion coefficient may be set to a negligibly small value i.e. of the order of 10^{-7}, this reduces the rate of movement of mobile biomass and so inhibits the spread of this local peak. If the mobile biomass concentration is below a threshold value the diffusion coefficient is high, and this results in the mobile biomass field reinforcing any local peaks. Mobile biomass is assumed to diffuse in the colony and the interaction between cytoskeleton elements and transport is encapsulated in a diffusion coefficient, D_n, which depends on local concentration of mobile biomass.

We assume the following simplified non-linear form:

$$D_n = \begin{cases} 10^{-7} D_b & n > n_0 \\ D_b & n < n_0 \end{cases}$$

The threshold concentration of mobile biomass, n_o, is set to a constant. Fig. 3.10 demonstrates the redistribution process and Fig. 3.11 summarises the recycling and redistribution processes within a fungal colony.

3.5.3.1 Redistribution definition

1. The diffusion coefficient for the mobile biomass field (n) is determined such that if n is below a threshold value the diffusion coefficient equals the D_n trait otherwise it is negligibly small value (10^{-7}).

2. The mobile biomass field is diffused and updated simultaneously across all cells with components derived from uptake and mobilization being added and components used for immobilization subtracted.

3.5.3.2 Redistribution Schematic

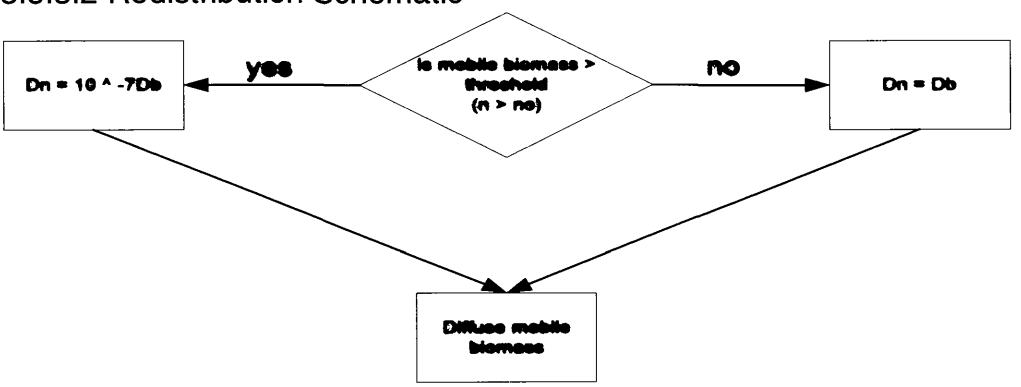


Figure 3. 10 Redistribution schematic demonstrating the computational stages associated with the transport of mobile biomass within the immobile biomass network

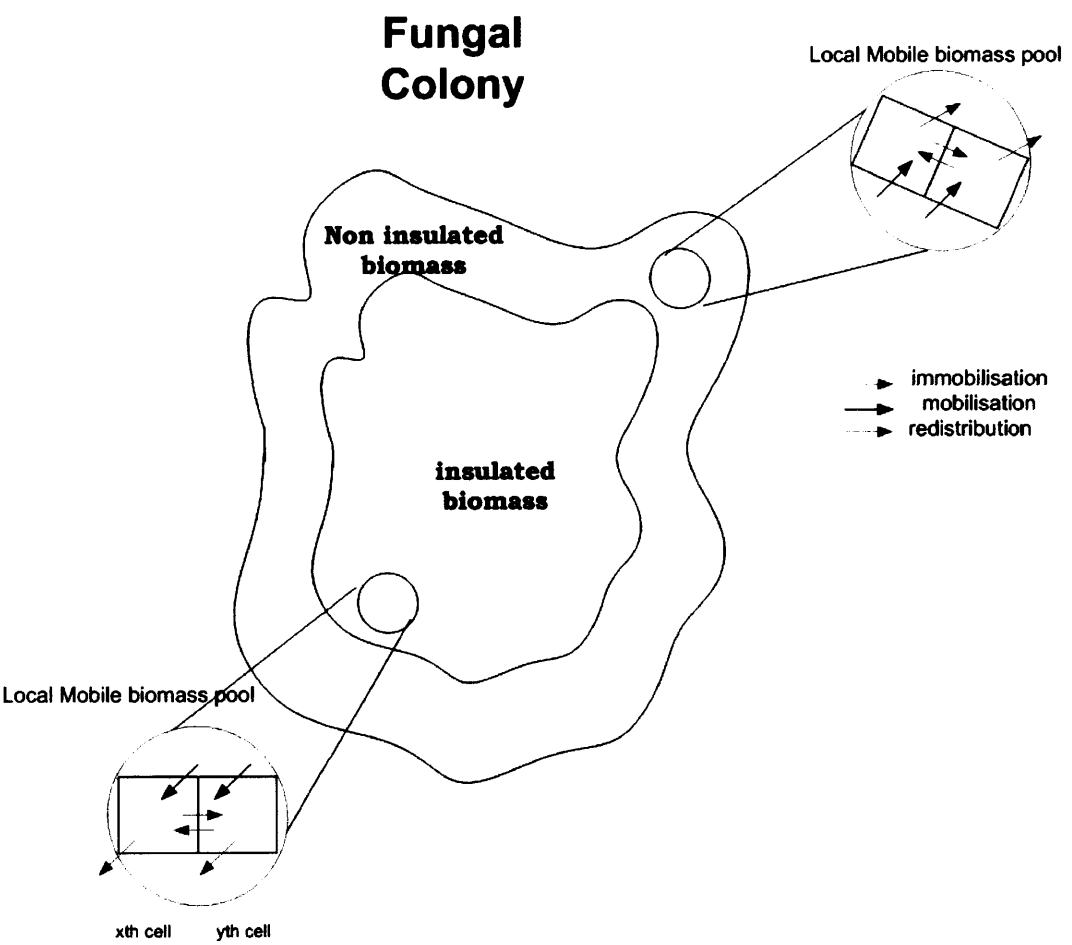


Figure 3. 11Colony representation depicting recycling of insulated and non-insulated biomass. This results in a net gain or loss to the local mobile biomass pool based on the traits for the mobilization and immobilization sub processes. The local biomass pool is then redistributed based on its diffusion coefficient which is determined by the local mobile biomass concentration. The figure shows the local processes that result in the local mobile biomass concentration for the xth and yth cells.

3.5.4 Growth

The non-insulated immobile biomass is increased by the immobilization of mobile biomass and reduced by the mobilized elements. At each time step a percentage of non-insulated biomass is converted into insulated biomass. This rate is species-specific, determined by the organism's trait values, and reflects insulating capabilities and apical extension. The insulated biomass also increases and decreases based on the immobilization and mobilization of insulated biomass processes respectively. The non-insulated biomass grows and is simulated using a passive diffusion mechanism as in the redistribution process.

$$\frac{\partial b_{ni}}{\partial t} = D_b \frac{\partial^2 b_{ni}}{\partial x^2} + D_b \frac{\partial^2 b_{ni}}{\partial y^2}$$

where b_{ni} represents the concentration of immobile non-insulated biomass

x and y are the variables representing two dimensional space

D_b is the diffusion coefficient

The diffusion coefficient controls the rate of spread i.e. the colony extension rate, and may be assigned a value in accordance with the underlying growth environment. For a homogeneous non-structured environment the diffusion coefficient is constant. However, for a structured environment such as soil the diffusion coefficient can be a function of porosity. If a site is a pore, growth can occur there. However, if the site is solid phase (aggregate) no significant growth can occur in this site. This can be achieved by setting the diffusion coefficient for a cell containing solid phases to a very small value. The figure below (Fig. 3.12) shows the distribution of D_b values based on a 2D thin section of soil. Where the diffusion coefficient is high there exists a pore and where it is low there exists a solid phase. Intermediate values for D_b represent cells that have some pore neighbours as well as some solid phase neighbours.

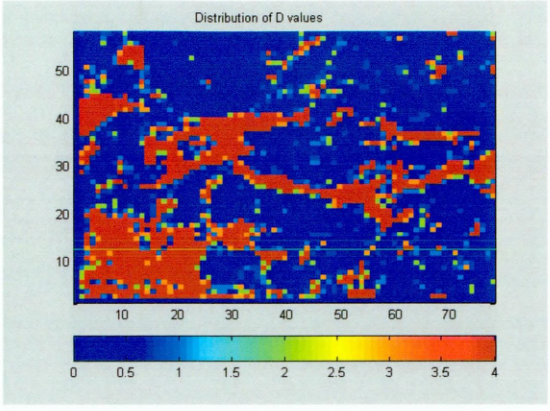


Figure 3. 12 Distribution of D_b values based on porosity values and number of pore neighbours, if cell is a pore and is completely surrounded by pore neighbours then it has a high diffusion coefficient (maroon). If the cell is a solid and has solid neighbours this translates to a low diffusion coefficient (blue).

3.5.4.1 Growth definition

For every cell that contains non-insulated biomass:

1. The percentage of non-insulated biomass that is to be converted into insulated biomass is determined as governed by a constant (ζ). This corresponds to apical growth of the hyphal tip, behind which becomes rigidified, insulated with time.

2. Adding and subtracting the derived immobilization and mobilization components respectively updates the non-insulated biomass field.

3. The insulated biomass field is updated to reflect the contributions from immobilisation and hyphal extent/rigidification (non insulated converted into insulated biomass ζ) and the loss due to mobilization.

4. The non insulated biomass diffuses as governed by the diffusion coefficient (D_b), which reflects the underlying environmental structure

3.5.4.2 Growth Schematic

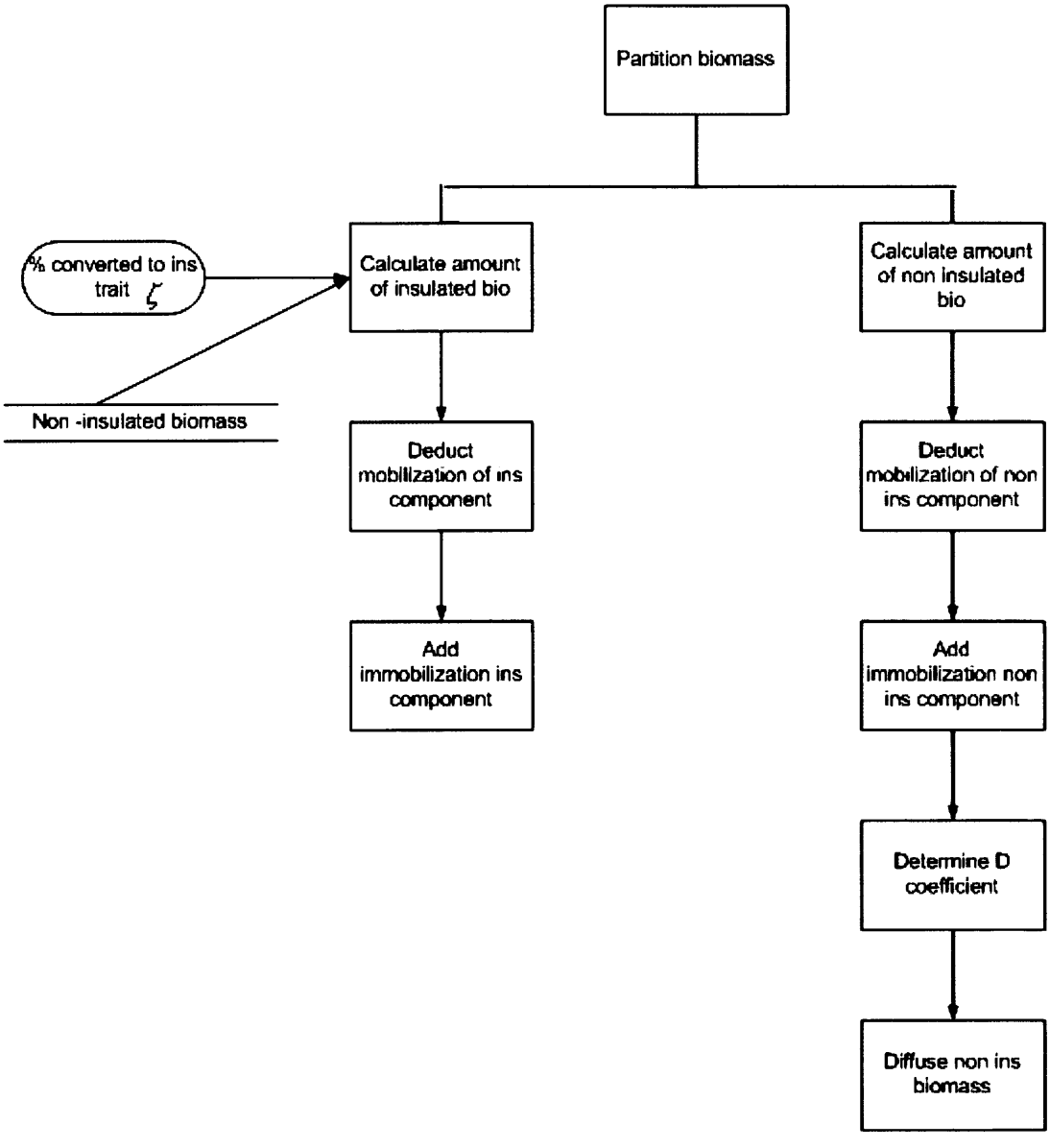


Figure 3. 13 Growth schematic, depicts the computational steps associated with the growth process

3.6 Environment

The environment comprises an underlying resource substrate and structure. Each site in the spatially discrete lattice has a number of associated characteristics associated with it. These are, existing resource level, replenishment rate and porosity. This allows a wide range of resource

levels and sites be arranged to provide homogeneous and patchy, heterogeneous distributions. Resource can also be added or replenished in space over time to understand the response of the colony to a dynamic environment. Varying the porosity values in space allows for a range of physical structural environments.

3.7 The mathematical model

We implement the above processes as a set of coupled state variables (fields): external substrate level, immobile insulated, immobile non-insulated and mobile biomass that correspond to the physiological processes described above and lead to a vector of ten traits:[α_n, β_n, α_i, β_i, γ, θ, λ_1, λ_2, D_b, D_n]. These parameters may be regarded as having a genotypic origin in the sense that they are constants that characterise functions, mapping environmental context onto mycelial phenotype. Each can, in principle, be directly measured. The mathematical model that describes the physiological processes described above is detailed in box 1. For simplicity the one-dimensional equations are shown but these equations are readily extended to two dimensions.

$$\frac{\partial b_i}{\partial t} = \zeta \left[\frac{\partial}{\partial x} D_b \frac{\partial b_{ni}}{\partial x} + \beta_2 \left(\alpha \pi^{\theta} - \beta \pi \right) b_{ni} \right] + \beta_2 \left(\alpha \pi^{\theta} - \beta \pi \right) b_i \quad \text{1.1 insulated biomass concentration}$$

insulated biomass = hyphal insulation + immobilisation of mobile biomass - mobilisation of non insulated biomass

$$\frac{\partial b_{ni}}{\partial t} = (1 - \zeta) \left[\frac{\partial}{\partial x} D_b \frac{\partial b_{ni}}{\partial x} + \beta_2 \left(\alpha \pi^{\theta} - \beta \pi \right) b_{ni} \right] \quad \text{1.2 non insulated biomass concentration}$$

non insulated biomass = diffusion of non-ins biomass + immobilistaion of mobile biomass - mobilistaion of non insulated biomass - hyphal insulation

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} D_n \frac{\partial n}{\partial x} - \beta_1 \left(\alpha \pi^{\theta} - \beta \pi \right) \left(b_{ni} + b_i \right) + \left(\lambda_1 b_{ni} + \lambda_2 b_i \right) r_e \quad \text{1.3 mobile biomass concentration}$$

mobile biomass concentration = diffusion of mobile biomass - immobilisation of mobile biomass + mobilisation of non insulated biomass + uptake

$$\frac{\partial s}{\partial t} = s_o - \left(\lambda_1 b_{ni} + \lambda_2 b_i \right) r_e \quad \text{1.4 external substrate concentration}$$

substrate concentration = initial concentration - uptake

key of symbols

$$\pi = \left[\frac{n}{b_{ni} + b_i} \right]$$

α and β are the immobilisation and mobilisation coefficients

β_1 and β_2 are efficiency conversion parameters; β_1 uptake -> mobile biomass; β_2 mobile biomass -> biomass

θ is the non-linear term associated with immobilisation

λ_1 and λ_2 are uptake traits for non insulated and insulated biomass respectively

D_b and D_n are the diffusion coefficients for non insualted and mobile biomass respectivley

b_{ni} and b_i are non insulated and insulated biomass concentrations respectively

n is the mobile biomass concentration

s is the substrate concentration

ζ is a constant which converts a percentage of non insulated to insulared biomass at each time step

r_e is external resource concentration

Box 3. 1The mathematical model see 3.2 for fuller descriptions of the processes.

3.8 Key Advancements of modelling framework

A limitation that obstructs more effective interaction between theory and experiment is the lack of an explicit physiological basis for the proposed processes in terms of parameters that can in principle be directly measured. Therefore, although models are formulated to link across scales, they often exploit arbitrary functions and parameter values, which are not directly measurable and so are generally limited to qualitative testing and only rarely explicitly incorporate physiological mechanisms. These models do not allow interpretation of macroscopic patterns in terms of observable microscopic mechanisms for a number of given contexts i.e. the link between genotype and phenotype through interaction with the environment. We are able to make this link and in Chapter 4 the link between genotype and phenotype in a simple environment is investigated. The model is first used to replicate colony forms obtained when grown on agar. In this scenario the environment is homogeneous in terms of resource and structure. The model results are used to identify which mechanisms are responsible for the emergence of structure within the colony.

Another major limitation of many of the theoretical approaches used to date is that they do not describe the capacity of fungi to reutilise their own hyphal material i.e. biomass recycling. Fungi are hypothesised to persist in dynamic and heterogeneous environments because of their capacity to take locally immobilised internal resources (e.g. those incorporated in structural or storage elements) and remobilise these into a form capable of being reutilised locally or directed to new internal sinks (e.g. sites of hyphal tip growth). This is one of the most important processes promoting indeterminate growth, but current theoretical approaches fail to embrace this process and thereby do not contribute to our understanding of fungal growth and development. This limitation is not immediately apparent from existing theoretical models as these models are applied in a relatively simple environmental context. These environmental contexts consider at the very most a heterogeneous non-structured environment even although most fungi reside in a heterogeneous environment with respect to resource and structure. The model is extended to 3D and coupled with a 3-D complex soil environment. The effect of the interaction of genotype and complex environments on phenotype is investigated (Chapter 5) and the results used to identify

which processes are responsible for survival in a complex heterogeneous environment with respect to structure and resource.

Finally very few approaches are designed to include the complex interactions that occur between colonies, and the effect this has on the distribution of biomass within individual mycelia and across the community. In Chapter 6 the model is extended to study the link between the dynamics of biomass distribution, community structure and function.

Chapter 4. Linking genotype to phenotype in an agar environment

4.1 Introduction

To validate the model formulation, generally we compare the phenotype of observed fungal colony forms with that predicted by the model. Here, we first explore the relation between genotype, the traits defining the fungal colony, and phenotype, the emergent growth pattern. The role of a more natural environment is considered in Chapter 5; for this investigation we maintain a simple homogeneous environment. A real-world analogue for a homogeneous environment is agar. Fungi grown on agar systems have been widely studied in the laboratory and agar has been used as a simplified environment for previously developed theoretical models (Bown *et al.* 1999; Lopez and Jensen 2002, Davidson *et al.* 1996, Boswell *et al.* 2003). There have been many experiments assessing fungal morphologies as a manifestation of the interaction between species and agar environment (Kennedy and Duncan 1995, Sturrock *et al.* 2002). Agar is a good experimental system as; first fungi are readily replicated on culture media; second they provide a homogeneous resource distribution for the colony to grow and this essentially constitutes a simple environment as there is no structural or biotic heterogeneity present; and last due to the ease with which individual environmental components such as temperature, light and water potential may be controlled. Keeping abiotic factors constant in natural systems is difficult therefore assessing what is responsible for a particular dynamic is impossible, but by keeping all but one factor constant the effect of that factor can be postulated.

By simulating a fungal colony interacting with an agar environment we can test the underlying assumptions of the conceptual model. Moreover we can investigate the effect of the interaction between genotype (the trait set) and simple homogeneous environment on phenotype and determine which processes are responsible for a particular phenotype characteristic.

4.2 The Model

4.2.1 Environment

An agar plate can be approximated by a two-dimensional (128 x 128 grid points) environment that is homogeneous in terms of resource and structure. The 2D environment can be replenished via a replenishment rate. This reflects the 3D nature of an agar plate with the surface being replenished with resource becoming available from lower regions of agar as the mycelium depletes the resource from the surface. Each cell in the environment can have low medium or high resource level.

4.2.2 Fungi

The model explores a range of growth forms resulting from different realisations (genotypes) of individuals and for different environmental contexts. By altering genotypic and environmental parameters separately, we probe the different contributions of genotype and environment to phenotype. The traits that control the uptake and recycling processes are varied and so we can investigate the interaction between processes and the resulting growth form for a given constant environment. By keeping the genotype constant and changing the context of the environment the effect of environment on growth form can also be investigated. In the following descriptions, $D_b = 10^{-10}$, $\xi=0.01$, $\lambda_2 = 0.01$, and $D_n max = 10^{-10}$. For ξ, we assume the rate of insulation of hyphae (% of non-insulated converted into insulated biomass) is constant among species. We also assume constancy in the surrogate signalling mechanism λ_2, controlling initiation of new hyphal tips from insulated biomass in response to availability of new resources. Finally, it is assumed that the maximum rate of diffusion of mobile biomass is comparable to that of biomass spread, allowing hyphal tips to be fuelled with resource necessary for apical extension. The precise value of the diffusion coefficient is obtained from the spatial and temporal scaling, which is based on the physical spatial and temporal scales of the simulated experimental system and the resolution of the spatial and temporal integration, i.e. the number of spatial and temporal grid points.

The environment is configured with initial data representing the inoculation of a small, active plug of mycelium placed into the environment. The inoculum is of the form:

$$b_{ni}(x,y)=\begin{Bmatrix} b_o, |d| \langle r \\ 0, \text{ otherwise} \end{Bmatrix}$$

where b_o is a constant representing the biomass level of the inoculum, d is the Euclidean distance of point $b_{ni}(x,y)$ to the proposed centre of the simulated inoculum and r is the proposed radius of the simulated inoculum.

No flux boundary conditions are imposed and the numerical calculations are carried out using the Crank Nicholson method in conjunction with Successive Over Relaxation (see Chapter 2 and appendix D for details).

4.2.3 Model Output

Model output is in a form that allows a numeric comparison of simulated and experimental results. Two-dimensional distributions of total biomass, i.e. mobile and immobile components, are plotted allowing examination of the spatio–temporal evolution of the system.

4.3 Results

4.3.1 Initiation of Growth

The model predicts a critical level of resource (1 unit per cell), below which the fungal individual displays a modest diffusive wave front exemplifying explorative behaviour. Above this critical level the wave front propagates autocatalytically, typifying exploitative behaviour. In both cases, wave fronts diffuse at the same rate and the quantity of biomass accumulated is consistent with literature (Bezzi and Ciliberto 2003). Three parameters were identified as being responsible for exploitative behaviour: the amount of resource in each cell, the biomass conversion efficiency (γ>0.2), and the

initial inoculum value. The subsequent form of the wave front is sensitively dependent on the values for genotypic parameters and the environment.

4.3.2 Impact of Genotype

In modelled systems where biomass conversion efficiency, γ, is low (0.2) and where mobilisation rate, β_n, is high (0.8), a ring-like form is produced (Fig. 4.1a). The colony centre has exhausted its resource, while the biomass on the periphery of the colony is exploiting newly encountered resource. Biomass in the centre of the colony is declining through remobilisation and is transported to the colony periphery. This type of behaviour is commonly observed in a number of different systems (Dowson *et al.* 1989). If γ is increased (0.8), then a plateau-like profile is obtained (Fig. 4.1b). There is still loss at each time step due to the biomass conversion efficiency parameter, but that loss is significantly less than in the previous simulation. The replenishment of external substrate offsets this loss, supporting further growth. If the replenishment parameter is set even higher a centrally peaked biomass distribution is obtained.

A qualitatively different wave front is obtained, using the same set of parameters to define the fungal individual as in Figure 4.1b, by increasing the degree of non-linearity in the immobilisation rate, θ. Instead of obtaining the profile in Figure 4.1b, defined concentric circles with some centralised local aggregations are obtained (Figure 4.1c). Rings are produced as a result of nonlinearities in the remobilisation apparatus in the following way. For $\theta > 1$, there is a switch from net mobilisation to net immobilisation when the mobile biomass concentration increases above a threshold (see 3.5.2). For high local uptake, the mobile biomass concentration will increase until there is a net immobilisation of biomass into new tip growth. This rapid increase in tip production leads to local depletion of external resource, and this in turn will cause the local mobile biomass concentration to decrease until it falls below the critical value and net mobilisation of biomass results. There will be a reduction in tip production and therefore uptake until the hyphae grow into a region of higher external resource when the process repeats. Central aggregations occur as a result of replenishment and through amplification of local uptake 'hotspots' by the same process.

By decreasing the mobilisation rate, β_n, and increasing the immobilisation rate, α_n, the switch point moves to progressively smaller values of the mobile biomass concentration. This results in progressively longer periods of immobilisation and shorter periods of mobilisation and so the rings of high biomass become thicker and more frequent until they eventually disappear (see Figure 4.1d, e and f). These concentric rings are exhibited by, for example, laboratory cultures of *Neurospora crassa* and *Streptomycetes rutgersensis* (Deutsch *et al.* 1993), and have been produced by other models such as Davidson *et al.* (1996), although the origin of the heterogeneities in terms of biological processes is not identified.

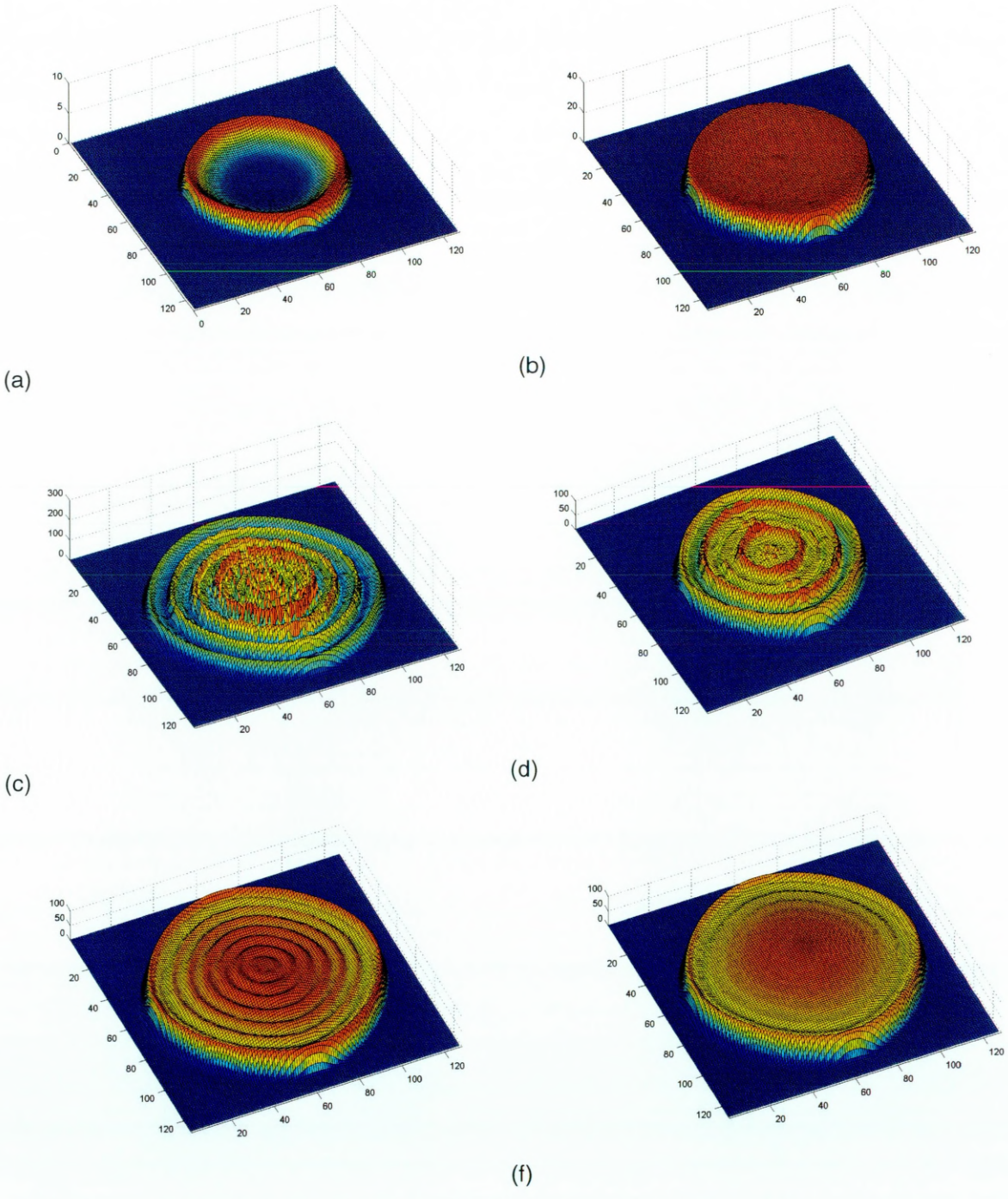
4.3.3. Impact of Environment

The macroscopic structure obtained is also sensitively dependent on the typically heterogeneous environment, where biomass recycling and nutrient reallocation according to local supply and demand becomes crucial. Figures 4.2 a, b and c illustrate a fungal individual growing in a heterogeneous environment. To focus on the effect of environment, we remove the non-linearity in the behaviour of recycling (setting $\theta = 1$) and set α_i=0.1 and β_i=0.9, so that there is net mobilisation of biomass as a result of recycling. This is consistent with biological processes such as autolyses, essential for long-term survival in heterogeneous environments. Initially, the fungus exploits the external resource located directly under the inoculum (Fig. 4.2a). Once a second resource 'hot spot' is located, investment into and consequent exploitation of that external resource is effected via reallocation of biomass (Fig. 4.2b). Finally, the fungal individual will grow out and explore from the newly found resource (Fig. 4.2c).

Figure 4.2d shows a fungal individual with the same genotypic parameters as Figure 4.1c, with external substrate replenishment rate ω=0. Here, the inner structure is heterogeneous, but the concentric rings are less defined and many more local hyphal aggregations are obtained. Heterogeneous and homogeneous profiles similar to those reproduced here have been obtained in laboratory experiments (Sharland and Rayner 1989a; Sharland and Rayner 1989b). With the parameter set of Figure 4.2d, but with the underlying resource at a much lower concentration, a

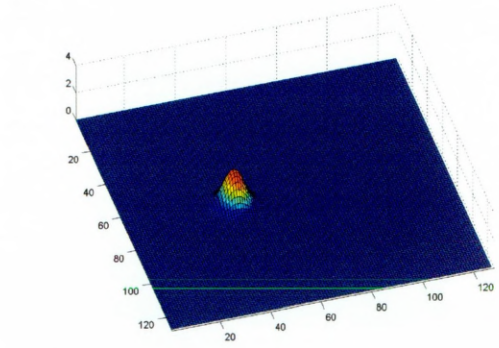
qualitatively different distribution of biomass is obtained with no local aggregations (Fig. 4.2e). This occurs because the external substrate, which is converted into mobile biomass and then biomass, is at a lower concentration. Therefore the effect of the non-linearities is small and the diffusive process can smooth any small variations out.

Figure 4. 1 Impact of fungal physiological traits on the emerged biomass profile

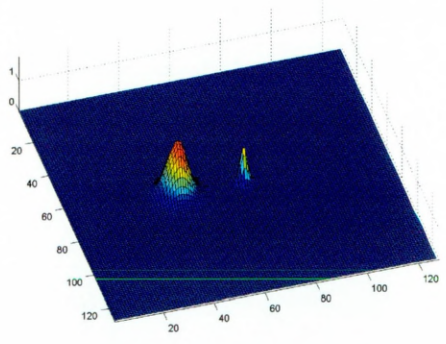


(a) α_n = 0.2, β_n=0.8, α_i = 0.5, β_i=0.5, γ 1=1.0, γ2=0.2, θ =1.0, λ1=0.95, λ2=0.01, D_b=10, D_n max=10, ζ=0.01, ω=0.01, time (t) = 20
(b) Same trait set as (a) but γ2=0.95, t=15
(c)Same trait values as (b) apart from θ and replenishment rate α_n = 0.2, β_n=0.8, α_i = 0.5, β_i=0.5, γ 1=1.0, (d) γ2=0.95, θ =3.0, λ1=0.95, λ2=0.01, D_b=10, D_n max=10, ζ=0.01, ω=0.1, t=15
(e) Same trait values as (c) apart from α_n and β_n - α_n = 0.4, β_n=0.6, α_i = 0.5, β_i=0.5, γ 1=1.0, γ2=0.95, θ =3.0, λ1=0.95, λ2=0.01, D_b=10, D_n max=10, ζ=0.01, ω=0.1. t=15
(f)Same trait values as (d) apart from α_n and β_n - rate α_n = 0.5, β_n=0.5, α_i = 0.5, β_i=0.5, γ 1=1.0, γ2=0.95, θ =3.0, λ1=0.95, λ2=0.01, D_b=10, D_n max=10, ζ=0.01, ω=0.1, t=15 See section 4.3 .2 for fuller explanations of the traits

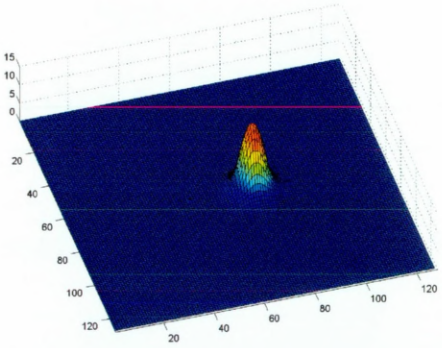
Figure 4. 2 Impact of the environment on emerged biomass profiles



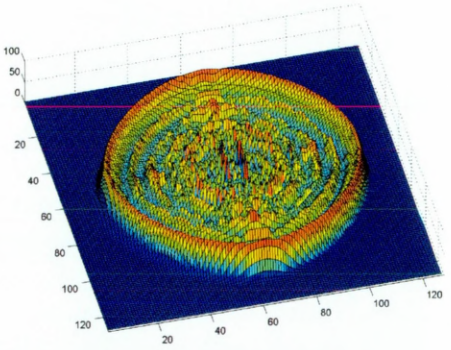
(a)



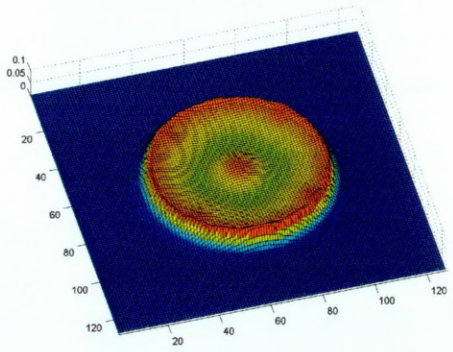
(b)



(c)



(d)



(e)

a) Fungal individual in heterogeneous environment α_n = 0.8, β_n=0.2, α_i = 0.1, β_i=0.9, γ 1=1.0, γ2=0.4, θ =1.0, λ1=0.95, λ2=0.01, D_b=10.0, D_n max=10.0, ζ=0.01, ω=0.01, time (t) = 10

b) Fungal individual in heterogeneous environment α_n = 0.8, β_n=0.2, α_i = 0.1, β_i=0.9, γ 1=1.0, γ2=0.4, θ =1.0, λ1=0.95, λ2=0.01, D_b=10.0, D_n max=10.0, ζ=0.01, ω=0.01, time (t) = 50

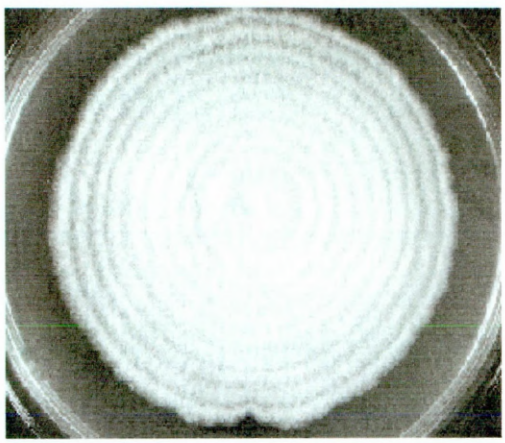
c) Fungal individual in heterogeneous environment α_n = 0.8, β_n=0.2, α_i = 0.1, β_i=0.9, γ 1=1.0, γ2=0.4, θ =1.0, λ1=0.95, λ2=0.01, D_b=10.0, D_n max=10.0, ζ=0.01, ω=0.01, time (t) = 70

d) Fungal individual has the same trait set as in Fig. 4.1(c) but ω equals zero, t=15

e) Fungal individual has same trait set as in Fig. 4.2(a) but the amount of resource in each cell is substantially less, t=30

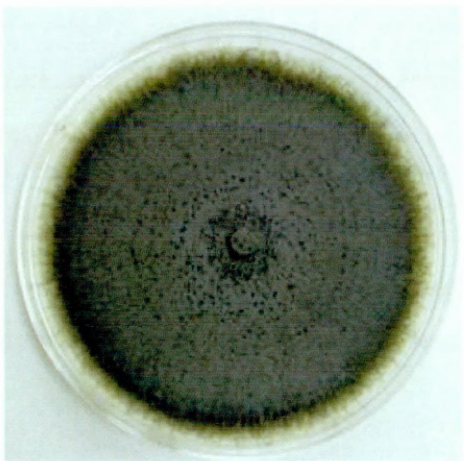
See section 4.3.3 for fuller descriptions of traits

Figure 4. 3 Observed colony forms



a) Observed colony form of *Sclerotina fructigena* (from Lysek 1984) similar to 4.1(e)

b) Observed colony form of *Phytophthora* spp. (from Kennedy and Duncan 1995) similar to 4.1(c)



c) Observed colony form of *Phytophthora* spp. (from Kennedy and Duncan 1995) similar to 4.1(b)

4.4 Discussion

Many growth forms that have been reproduced in more phenomenological models e.g. (Davidson *et al.* 1996) can be reproduced here, and these forms emerge from interactions among genotypic parameters relating to biomass recycling (Fig. 4.1) and the environmental context of the mycelium (Fig. 4.2). The model output is also consistent with observed colony forms i.e. Figure 4.1(e), (c) and (b) simulate the observed colony forms of 4.3(a), (b) and (c) respectively.

The internal structure (local aggregations and concentric rings) is caused by the interplay of mobilisation and immobilisation processes and the transport of mobile biomass. Local regions within the mycelium immobilise or mobilise biomass based on the local mobile biomass concentration. Concentric rings feature in many other dynamic, complex systems such as the B-Z reactions and bacterial colonies (Stewart 1997, Kreft *et al.* 1998). Such patterns are likely to arise in any dynamical system which possess certain features. These features include: spatial extension, an oscillatory dynamic and coupling rules for how one region influences its others (Stewart 1997). Indeed, these features are incorporated in the conceptual model and it can be considered a dynamic, complex system.

The equations of box 1 (Chapter 3, Section 3.7) reflect the complexity that is exhibited by many fungal colonies by incorporating cooperation, self-organisation, emergence and non-linearities, and these are key features of complex systems. Cooperation is mediated through the transport of a mobile biomass field allowing distal regions to 'communicate' via transport of mobile biomass. Mobile biomass is extracted from areas that are not undergoing exploitation and transported to regions undergoing exploitation via the diffusion process.

Self-organisation in fungal systems is exemplified by a switch from exploitative to explorative behaviour. This is achieved in the model by the mobile biomass concentration (n), once it drops below a threshold value the local response of the mycelium changes from being exploitative by inhibiting diffusion of the local mobile biomass which results in more biomass production and this in turn causes more local uptake to exploratory behaviour by diffusing the mobile biomass in order to promote growth in new areas.

Since the complex colony patterns are produced as a consequence of the interaction of simple rules, and algorithms do not encode the colony-scale patterns then the structures are emergent phenomena. Like Conway's Game of life given the initial conditions and the rule set underlying the organisation of fungal systems the colony form/pattern is unpredictable. Emergent properties are still a logical result, just not a predictable one.

Finally a characteristic feature of the vast majority of complex systems encountered in nature is the fact that they are nonlinear. The apparent complexity of fungi cannot therefore be described by linear equations/relationships alone. The equations prescribe a nonlinear dependence of local biomass assimilation on the ratio between mobile biomass and immobile biomass concentration. This nonlinear dependence allows a rich variety of complex colony forms to be simulated. Non-linearities often characterise regulatory interactions i.e. those that switch a process on or off depending on the signal.

The developed model for the phenotype of fungal mycelia incorporates, for the first time, the processes associated with recycling and transport of biomass that are fundamental to indeterminate organisms. Observed colony-scale features are reproduced and can be interpreted as emerging from relatively simple interactions between localised processes governing recycling of mobile biomass, and colony-scale transport. Broad ranges of observed phenotypes arise from different realisations of these processes as characterised by different associated parameters and from different environmental contexts. Recycling of biomass is described by rules that implicate only the local, internal conditions in the mycelium. Coherence and symmetry of patterns at the scale of the mycelium are mediated by subsequent transport of mobile biomass that result from the recycling process. Symmetry is lost when assumed non-linearities in biomass recycling become dominant. Such non-linearities might have their origins in regulatory processes affecting the immobilisation of mobile biomass for the local production of biomass. The model shows how local, hyphal scale processes and transport are involved in the production of colony-scale features. Clearly, there are many processes and features of real fungi that have been ignored in the current implementation. Notwithstanding this, the results here show that apparently complex behaviour can result from simple local rules spatially mediated by internal transport, and additional sophistication will not affect this general conclusion.

Although agar systems are essential in validating the processes of the model they are far removed from the natural environment in which fungi would reside. Fungi reside in heterogeneous, patchy environments where species have adapted to survive in conditions often hostile to the majority of

organisms. Moreover, Rayner (1988) states "Growing fungal mycelia under controlled conditions on homogeneous, artificial laboratory media is hardly the best way to appreciate developmental plasticity. It is rather akin to incarcerating a human being in an environment with plentiful supplies of bread and water but no source of stimulation." An understanding of colony growth forms in their natural environments has potential to address many ecological questions. Chapter 5 investigates the colony growth forms resulting from an interaction between genotype and a complex soil environment.

Chapter 5. Linking genotype to phenotype in a complex environment

5.1 Introduction

The complex soil pore network consists of a labyrinth of pores, pore throats and small connecting channels, and is responsible, in part, for a range of bio-available spatial scales. The spatial distribution of pores and pore throats governs the connectivity of the pore network, which in turn regulates the movement of gases, liquids and many microbes. In particular for fungi, the air filled pore volume is a significant factor in dictating mycelial spread in soils, with reduced fungal growth where air-filled porosity is low (Otten *et al.* 1999).

As well as the physical architecture of soil the distribution of resources also governs the dynamics of the fungal mycelium. Throughout the pore network, resources will be heterogeneous in terms of distribution and bio-availability. However, after rainfall dissolved organic molecules are flushed from plant surfaces and surface litter, and this provides a relatively homogenous carbon environment throughout the pore network, albeit temporarily. In addition to structural and resource heterogeneity the distribution of microbes affect the colony dynamics via biotic interactions. These heterogeneities lead to a complex environment in which the fungal colony must persist. Fungi have evolved a number of physiological and morphological adaptations allowing colonization of the heterogeneous terrestrial environment. As fungi grow through soil they respond to their local environment by adjusting rates of uptake, growth and biomass recycling, for example: by exponential growth upon encounter of fresh resource; production of fruiting bodies when the environment becomes hostile; recycling of the mycelium once the resource in the local environment has been fully utilised. Furthermore, as fungal hyphae are just a few micrometers in diameter, they can pass through small channels connecting clusters of pore spaces. Any resource located in this otherwise unavailable pore space may be utilised, and as fungi can redistribute nutrients and biomass internally through the fungal network, such resource may be used by other parts of the colony and hence by other biota. Uptake of external resource from and growth through the environment are central to the development of most living organisms. Additionally, for fungi,

biomass recycling, referred to as recycling hereafter, is hypothesised to be crucial for survival in complex environments such as soil. Other indeterminate systems such as coral and lichen rely on recycling as a key survival process (Lesser 2004) and (Ellis *et al.* 2005). Deacon (2005) states "that fungi recycle as much of the 'aged' mycelium as possible" and Carlile *et al.* (2001) recognise that redistribution and degradation of old mycelium occurs, but state that "little is known about the processes responsible for this". Fungi growing in soil are challenged by the efficient foraging of patchy resource and the economical use and redistribution of this resource for metabolism or biomass generation (Boddy 1999). Inefficient redistribution of resources and biomass may restrict growth from one resource patch to the next. Such a scenario would eventually exhaust colony growth, thereby limiting resource exploitation in space, which may ultimately threaten the persistence of the individual. Although biomass recycling is generally acknowledged as a key process enabling fungal colony survival, particularly within heterogeneous or low nutrient environments, any understanding of how this relates to function is scant.

Here, we consider modelling colony growth in soil. The model is extended to three dimensions and a combination of modelled soil structure derived from experimental data and a range of genotypes with different recycling strategies is used to explore the impact of biomass recycling on fungal colony growth in complex environments.

To measure the relation between biomass recycling and colony growth a fitness metric is required which allows a comparison of the responses of different genotypes. The definition of fitness for filamentous fungi is a contentious one. There are many components of fitness that ultimately contribute to survival and reproduction of a fungal individual. These components may be associated with various stages of the fungal lifecycle such as, spore production and dispersal, colonisation and exploitation and reproduction. Fitness components relating to the colonization and exploitation phases include resource exploitation, combativeness and environmental tolerances (Brasier 1999). We further acknowledge that fungal colony dynamics are affected by biotic heterogeneities and note that the formulism here does not address this. Importantly, as the modelling approach used here deals only with vegetative growth of a single colony focusing on a single aspect of the life cycle, i.e. resource exploitation, foraging capacity, is sufficient (Pringle and

Taylor 2002). Assuming external resource is converted and allocated, with some efficiency, into hyphal biomass, the biomass production rate (units of biomass per volume per unit time) is an appropriate foraging measure referred to as fitness hereafter. This metric encompasses the spatial and temporal extent of a single colony.

Here, a study similar to Stacey *et al.* (2001), but using a different modelling approach, is initially carried out to determine whether finite colony expansion occurs in three dimensions. Stacey *et al.* (2001) demonstrate in two dimensions that finite colony growth occurs when the proportion of randomly removed resource sites (removed with probability *p*, resulting in a prescribed resource density) falls below a threshold value (resource density threshold). Otten *et. al.* (2004) also shows that the level of resource at each site affected this threshold value. We construct model scenarios to target the following questions:

1) Are the two-dimensional results of Stacey *et al.* 2001; Otten *et al.* 2004) observed here in three dimensions:
    a. Do resource density thresholds that cause finite colony expansion exist in 3D and what is the effect of resource density on colony fitness;
    b. Does resource level affect these threshold values;
2) Does biomass recycling affect these thresholds and hence fitness;
3) What are the consequences of biomass recycling in complex environments on fitness?

5.2 Methods

The simulations described comprise a novel combination of environment and organism. The physical structure of the environment is derived from a field soil sample via Computer Aided Tomography (Feeney *et al.* 2006). Varying the amount and distribution of resource within this structure generates different environments. The growth of a fungal colony through the range of environments is simulated using the modelling approach described here. Changing the physiological trait values used in each simulation allows growth modelling of different fungal colonies.

5.2.1 Environment

Environments may be homogeneous or heterogeneous in both structure and resource distribution. The size of all environments is 50x50x50 cells and all possess a physical structure determined by

the pore network, the distribution of pores and solid phases within the soil structure. The colony may grow through pores; growth is constrained by solid phases. Pores vary in size and the network may range from being entirely porous (no solid phases) to a labyrinth of pores and pore throats. The pore network is seeded with different resource distributions at a prescribed resource level. All simulations run for sufficiently many iterations i.e.1000 iterations.

5.2.1.1 Structural Heterogeneity

A homogeneous structure is a uniform pore network and so imposes no spatial constraints, up to the size of the environment, on the fungal colony. Heterogeneity is introduced into the structure in accordance with the pore network specified in the soil sample. The soil sample measures 892^3µm and is processed to obtain a volume of 200 pixels in each dimension (see Feeney *et al.* 2006) for details. From this volume, a sub-volume of 50 pixels in each dimension is extracted here and tested to ensure that a connected pathway exists through the porous network (Fig. 5.1). All simulations with a heterogeneous structure, referred to as structure hereafter, use this pore network.

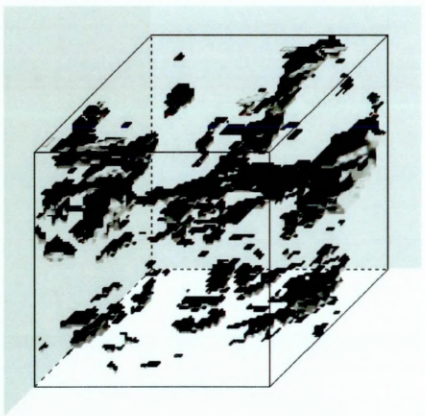
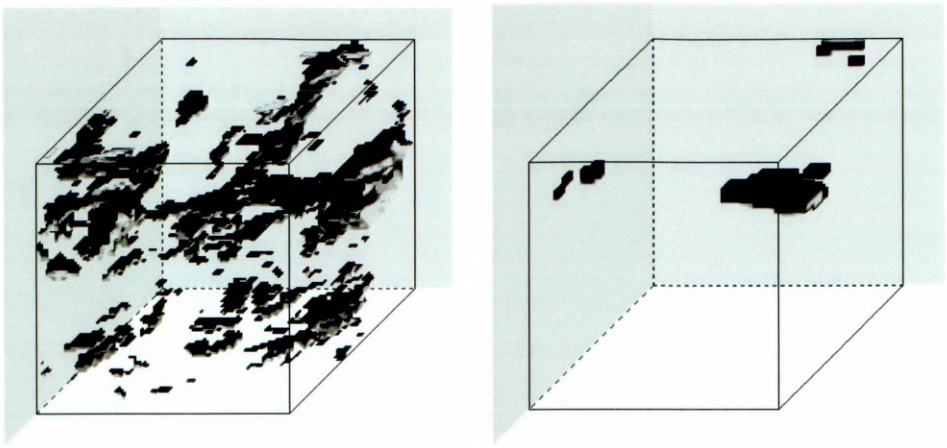


Figure 5. 1 3D visualisation of extracted soil structure: black represents pore space; white solid aggregate. This pore network is used in all simulations with a heterogeneous structure

5.2.1.2 Resource Heterogeneity

In a homogeneous resource distribution, resource is distributed uniformly within the pore network (Fig. 5.2a). In a heterogeneous resource distribution, resource is distributed in patches, hereafter termed hotspots, within the available pore network for example (Fig. 5.2b). Varying the number, size and locations of these hotspots allows different realisations of resource heterogeneity. Where colony growth in heterogeneous and homogeneous resource environments is compared the total amount of available resource is identical.



(a) homogeneous distribution **(b) heterogeneous distribution**

Figure 5. 2 3D visualisation of extracted soil structure with a homogeneous and heterogeneous resource distribution.

5.3 Scenarios

5.3.1 Do resource density thresholds that cause finite colony expansion exist in three dimensions?

(a) The effect of resource density on fitness (no structure, heterogeneous resource distribution)

To construct different resource densities we initially consider all sites in the volume to contain a prescribed amount of resource (R =0.05). Subsequently, these resource sites are removed randomly with probability 1- *p* and so will contain resource with probability *p.* Different resource distributions are obtained by varying *p* from 0.01 to 0.97 in steps of 0.04. In all simulations, the environment is inoculated with the fungal colony at its centre, consistent with Otten *et al.* (2004) and Stacey *et al.* (2001), and the time taken for the colony to reach the first of any of six edges determines the crossing time. Individual fitness is defined as biomass produced until the crossing time divided by crossing time. This provides a measure of fitness related to the rate and extent of resource exploitation as discussed above. The fungal individual used here, in (b) and in 5.3.2 is as detailed in Fig. 4.2(a-c) of thesis section 4.3.3 and Falconer *et al.* (2005), and is chosen since previous work regarding the interaction of genotype and environment exists for this individual and the genotype has recycling capabilities hitherto unexplored. In this scenario, however, the individual's recycling parameters are set to zero, leading to the trait set: [$\alpha_n = 0.8, \alpha_i = 0.0, \beta_i = 0.0,$ $\theta = 1.0,$ $\lambda_1 = 0.01$ and $\lambda_2 = 0.95$].

(b) the consequence of resource level on fitness (no structure, heterogeneous resource distribution)

Fitness and resource density threshold values for each of the substrate levels (R = 0.05, 0.1 and 0.15) are evaluated. For each site containing resource, the level of resource is set to R. The same resource distributions for each probability level are generated as in 5.3.1 (a). To inform the selection of the lowest level of substrate, an investigation to identify the minimal substrate level to support colony expansion was undertaken. The minimal level of 0.02 was found by progressively reducing substrate levels in simulations until finite colony growth was observed with the highest resource density. The value of 0.05 is chosen as the poorest substrate level, above but close to this minimal level. Additional intermediate vales of 0.1 and 0.15 were introduced to determine fitness in higher resource levels.

5.3.2 Does biomass recycling affect fitness and resource density thresholds in three-dimensions (no structure, heterogeneous resource)?

The simulations with resource level (R = 0.05) of 5.3.1 (a) are repeated using the same resource density realisations. Here the fungal individual has recycling capabilities with genotype: [α_n = 0.8, α_i = 0.1, β_i = 0.9, θ = 1.0, λ_1 = 0.01 and λ_2 = 0.95]. Unlike the individual simulated in 5.3.1 (a) the recycling parameters (α_i = 0.1, β_i = 0.9) are non-zero. These parameters increase the rate of mobile biomass production. They control the rates of inter-conversion from mobile (α_i) and immobile (β_i) insulated biomass and, since $\beta_i > \alpha_i$ and θ =1.0, the amount of mobile biomass that is redistributed within the colony is increased. This allows assessment of the effect of recycling on fitness and resource density thresholds.

5.3.3 What is the consequence of biomass recycling in structured complex environments?

In these simulations a structured environment is used as described in 5.2.1.1 and the colony is introduced at the edge of the pore network rather than in the centre. The fitness value is evaluated as before, but the crossing time relates to the time taken for the colony to reach the opposite edge of the connected pore network, so maximising the size of the colony developed. A set of 100

different individuals is randomly generated. From this set, another 100 are created using the same parameters as in the original set, and here both recycling traits are assigned the value zero (α_i , $\beta_i = 0$).

Structured environment, homogeneous resource distribution

The effect of a heterogeneous structure (Fig. 5.1) and a homogeneous resource distribution on fitness for recycling and non-recycling individuals is investigated. In each pore the amount of resource is 0.859 and the total amount of available resource is 3470. This total resource level is derived from the lowest limit for R allowing colony expansion in (b)(i) below, and the amount in each pore is the total resource divided by the number of pores.

Structured environment, heterogeneous resource distribution

In this scenario the heterogeneous structure of 5.2.1.1 is used together with a number of different realisations of heterogeneous resource distributions providing a complex environment for the colony to grow. The different resource distributions are obtained by either keeping the positions of resource hotspots constant and varying the resource level as in (i) or by varying the positions and levels of the hotspots for a constant (total) level of resource as in (ii). The effect of biomass recycling on fitness in these different environments is investigated.

(i)Resource level

Using the resource distribution of Fig. 5.2 (b) a comparison of fitness at each of the substrate levels (R =10.5, 10.75 and 11.0) is conducted. The selection of the lowest substrate level was determined by identifying the maximum resource level that caused finite colony expansion and hence poor fitness values for non-recycling individuals (R=10.5, total resource = 3470). The resource level is increased in increments of 0.25 up to R=11.0. The substrate levels are much higher than those of 5.3.1 (b) as in this scenario there is the additional complexity of physical architecture. This architecture constrains the locations of available resource sites, and so more resource is needed at each site to overcome finite colony expansion.

(ii)Positions of resource hotspots

The location and size of the hotspots are randomly determined, and 10 different realisations of resource distributions are used. The total amount of available resource is the same in each case. From each of the 10 different resource distributions, the fittest 20 individuals (top 20 list) of the

original 200 (100 with and 100 without recycling) are selected. The number of times an individual features in all 10 top 20 lists is determined.

5.4 Model Output

The model output consisted of statistics recording the fitness value of a colony as a function of resource level and trait values. A Principal Component Analysis was undertaken to determine whether the trait set could be collapsed into a smaller subset reducing the dimensionality of the data. The results of this indicated that the dimensionality cannot be reduced as the first two components attributed to only 49.2% of the variation.

5.5 Results

5.5.1 (a) Resource density thresholds exist in three-dimensions causing finite colony expansion and reducing fitness.

Fitness increases with an increase in the probability that a site contains resource (Fig. 5. 3). The graph is sigmoidal, possessing a lower threshold, p_l= 0.37, below which fitness is approximately zero corresponding to finite colony expansion at low resource density. Over time the colony becomes insulated corresponding to rigidification of hyphal tubes, and since insulated biomass does not diffuse this prevents further resource exploitation and exploration. The colony does not form connections among resource hotspots and does not reach the opposite edge by the maximum number of iterations (1000), resulting in a low fitness value. An upper threshold p_u = 0.93 above which there is no increase in fitness also exists. The fitness value becomes saturated as above the upper threshold the extra resource sites have a minimal impact on resource exploitation. This is because most of these extra resource sites do not lie on the connected path from the inoculum to the connected plane edge.

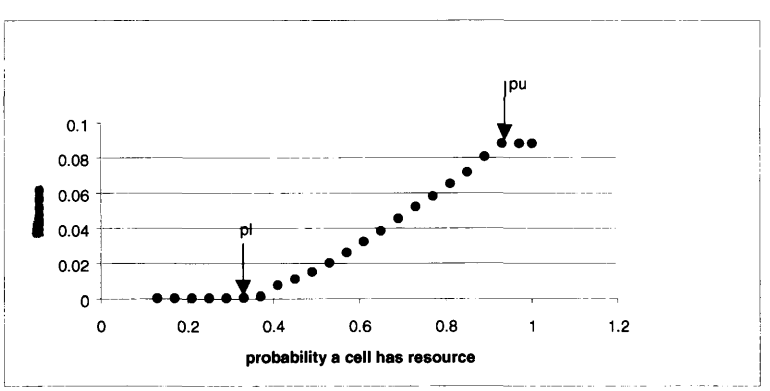


Figure 5. 3 effect of resource density on colony fitness, the more resource sites available the fitter the colony.

5.5.1 (b) Resource level of sites affects resource density thresholds and fitness

Increasing resource level promotes an increase in fitness (Fig. 5.4). As fitness is derived from resource exploitation, if there is more resource to exploit this then has a positive effect on fitness. The upper threshold, $p_{u,}$ is the same for R=0.05, 0.1, 0.15 as p_u is a consequence of the location of the resource sites, which remains constant among resource levels as the same random number sequence is used. This is not true for the lower threshold, p_l. Decreasing the resource level increases the lower resource threshold (p_l), leading to a greater probability of finite extension and therefore decreasing fitness. If the colony ceases to expand, this affects its ability to forage for other available resources decreasing fitness.

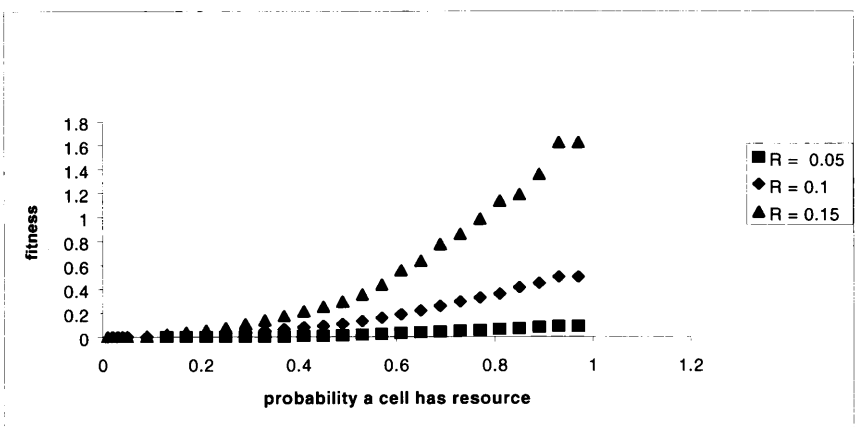


Figure 5. 4 effect of resource level on fitness, as increase the amount pf resource in each cell the fitness increases

5.5.2 Biomass recycling lowers resource density thresholds, which increases fitness

Table 1 summarizes the effect of biomass recycling on resource density thresholds for different resource levels (R). For all resource levels recycling decreases the lower threshold below which fitness tends to zero due to finite colony expansion. Recycling aids mycelial connections among patchy and limited resources thus increasing the associated fitness. The impact of recycling increases as resource density increases (Fig. 5.5).

R	Threshold with recycling	Threshold without recycling
0.05	0.33	0.37
0.1	0.17	0.21
0.15	0.05	0.09
0.5	0.01	0.02

Table 5. 1 Effect of resource level and biomass recycling on resource density thresholds

A paired sample t-test on sample populations of individuals with and without recycling apparatus for each R indicates that recycling had a significant impact on fitness. The **p** value is the probability that the means or variances of the two samples are the same and, since **p** = 0.00, recycling has a significant impact on fitness values.

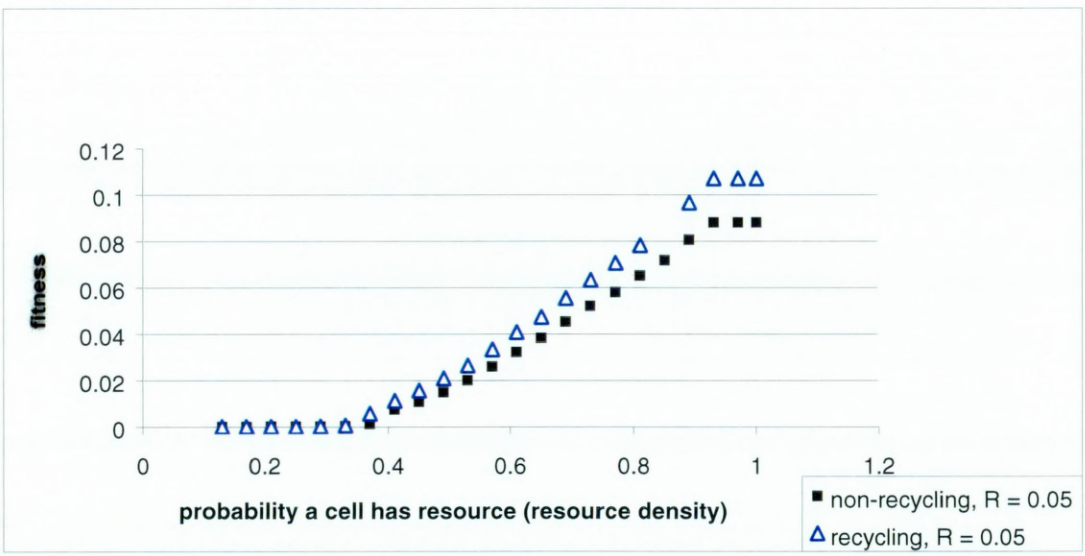


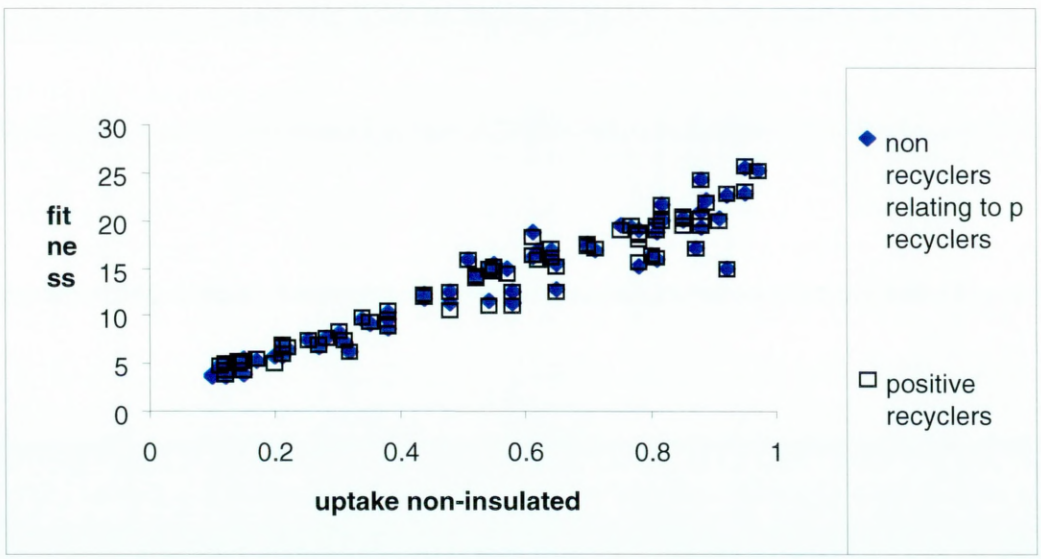
Figure 5. 5 Effect of biomass recycling on fitness and spatial threshold

5.5.3 a) Biomass recycling is not related to fitness in a structured environment with a homogeneous resource distribution

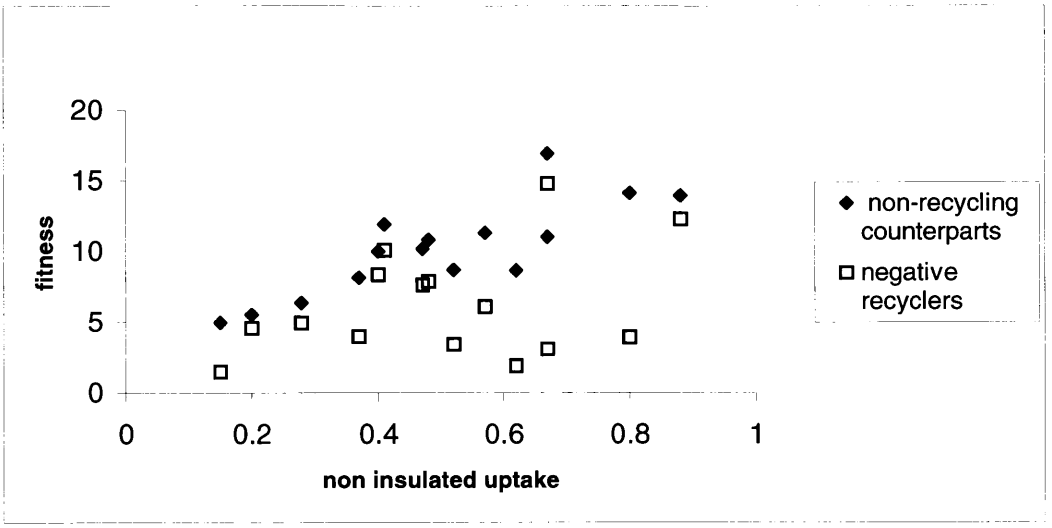
In the creation of the 100 individuals with recycling apparatus (5.3.3) there are no rules imposed on the relation between the rate of immobilization (α_i) and mobilization (β_i) of insulated biomass. The random number generation of trait values therefore produces two groups of individuals: $\beta_i >= \alpha_i$ and $\beta_i < \alpha_i$. In the first case, termed positive recyclers, individuals are able to convert immobile

biomass into mobile biomass for use in other areas of the mycelium via recycling. In the second case, termed negative recyclers, individuals assimilate biomass at a faster rate than it may be remobilised. Consequently there is no net production of mobile biomass and this precludes recycling. In our analysis we first compare the fitness values of positive recyclers and their non-recycling counterparts (those individuals with the same traits except β_i , $\alpha_i \neq 0$). We then compare the fitness values of negative recyclers and their non-recycling counterparts.

A paired sample t-test on the fitness of the positive recycling group and their non-recycling counterparts (Fig. 5.6(a)) indicates that positive recycling does not have significant impact on fitness (p=0.88). This is because, in a homogeneous resource distribution, resource is located in each cell and connections between resources are not necessary. Therefore recycling is a redundant process. A paired sample t-test on the fitness values of the negative recycling group and their non-recycling counterparts (Fig. 5.6(b)) indicate that negative recycling group are significantly less fit (p=0.00) than their non-recycling counterparts.



(a)



(b)

Figure 5. 6: effect of biomass recycling in a structured environment with a homogeneous resource base for (a) positive recyclers and (b) negative recyclers .

5.5.3 b) Biomass recycling increases fitness in complex environments with patchy resources

(i) Effect of resource level in structured environment with heterogeneous resource distribution.

As resource level, R, increases, the fitness of both recycling and non-recycling individuals increases (Fig. 5.7). For the non-recycling group there exists a lower threshold (R = 10.75) below which all non-recycling individuals do poorly (Fig. 5.7(a)). Some non-recycling individuals are not fit irrespective of the level of resource. Note that this is also true for individuals with recycling capabilities, and these individuals correspond to negative recyclers discussed in 5.3.3 (a). All further analysis will be carried out using positive recyclers only as it is reasonable to assume that in natural systems negative recyclers will have been selected against. As the level of resource increases the difference between fitness for recycling and non-recycling populations decreases becoming non-significant. This is supported by the results of a paired sample t-test p = 0.000, 0.001 and 0.378 for R=10.5, 10.75 and 11 respectively.

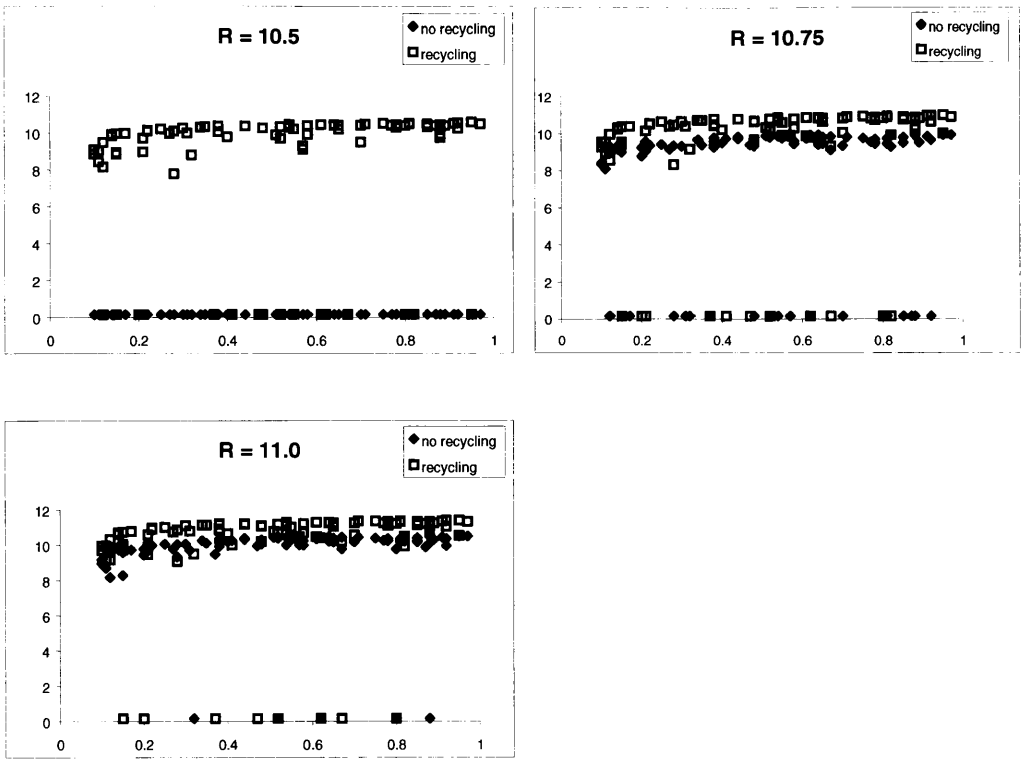


Figure 5. 7 effect of resource level on fitness for recycling and non-recycling individuals in heterogeneous environment in terms of resource and structure.

Table 2 shows the average crossing time (for all individuals) for recycling and non-recycling populations growing in varying resource levels (R) in a structured environment with a heterogeneous resource distribution. Recycling individuals are fitter as they grow faster, reaching the opposite edge of the pore network more quickly. Note the difference between recycling and non-recycling individuals reduces as R increases.

	Crossing time	
R	Recycling	Non-recycling
10.5	477	1000
10.75	419	504
11.00	334	395

Table 5. 2 Average crossing times and biomass production for recycling and non-recycling individuals

(ii) Effect of location of hotspots (different resource realisations)

The number of times an individual features in the top 20 fittest list is plotted (Fig. 5.8). Recycling capabilities are highlighted on the plot. Individuals with recycling are fittest over a larger number of

environments than non-recyclers. For the non-recyclers that were in a top 20 list for a given environment the corresponding recycling individual also featured in the top twenty list. It is only individuals that recycle biomass that are fit in six or more different environments.

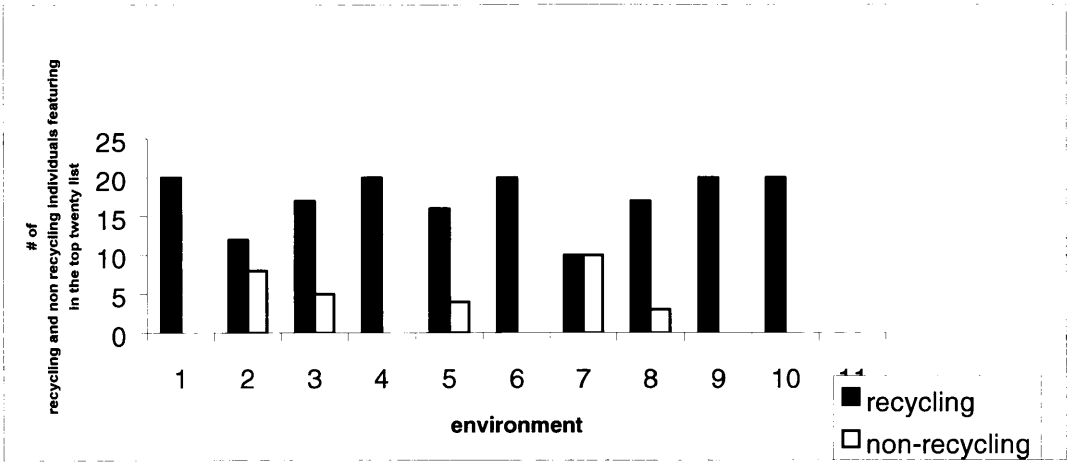


Figure 5. 8 plot showing which individuals are the fittest over a number of environments with different resource distribution

5.6 Discussion

Resource density thresholds exist based on the number of randomly removed resource sites, causing finite colony extension in a non-structured, resource patchy, three-dimensional environment. This threshold (0.37), corresponds to 63% of sites being resource free (for a resource level R = 0.05), and is consistent with percolation thresholds of 3D media (0.311). The percolation threshold decreases with dimensionality as higher dimensionality offers more ways to get around an unoccupied region. Fitness increases with a corresponding increase in resource density and/or resource level, since in either case an increase allows more resource to be exploited by the colony. Finite colony expansion occurs when growth from one resource site to the next is not possible, as the distance/amounts between/of resources is too large/small and connections among them cannot be achieved. Finite colony expansion leads to an unfit individual in terms of resource exploitation, as it does not have the capacity to exploit other resources available in the environment. Over time the colony will become insulated and common macroscopic manifestations of insulated biomass include the formation of fully anastomised centres of colonies in nutritionally depleted environments. This insulated biomass does not diffuse, and so foraging capabilities are reduced.

Moreover, the insulated biomass will decay over time, with no new uptake, resulting in non-continuance of the colony.

Recycling lowers the resource threshold value for finite colony expansion allowing individuals to persist in non-structured environments with patchy resource distributions. The persistence of an individual allows it to gain access to other resource therefore increasing the fitness of the individual. Recycling converts aged, insulated biomass into mobile biomass (n) at a rate governed by β_i, which is then directed towards regions of the colony undergoing exploitative phases. This redirection emerges from a non-constant diffusion coefficient for mobile biomass (D_n) as detailed in Chapter 3. Essentially recycling increases the rate of uptake by non-insulated biomass on the periphery of the colony. These regions are being fuelled with mobile biomass that become immobilized non-insulated biomass at a rate governed by α_n. Consequently this increases the rate of uptake, which in turn increases the local non-insulated biomass concentration. If the non-linear term (θ) associated with the non-insulated immobilization rate is greater than 1 this further enhances the autocatalytic effect. The increase in non-insulated biomass at the exploitative boundaries of the colony allows connections among resource patches that otherwise would not have been possible.

In the case of a structured environment (pore network) with a homogenous resource distribution resource is allocated to each cell in the pore network. Since resource exists in each cell no connections among resource hotspots are required and so recycling is not a crucial process in this context. Recycling is, however, crucial for environments with a heterogeneous resource distribution within a pore network. The complexity of the pore network will further constrain connections among resource patches distributed within the pore space. It was demonstrated that two types of recycling existed: those that had a positive effect and those that had a negative effect on colony fitness. A negative recycling capability arises from the random generation of trait sets here, although it is unlikely to exist in natural systems with selection pressures. Where recycling is positive, this ability provides more effective redistribution of biomass when resources are patchy and limited, and this allows the fungal colony to grow faster over nutrient free regions. Fast growth in nutrient free

regions is typical of explorative behaviour exhibited by fungi in heterogeneous environment (Boddy 1998). When resources are low and patchy, biomass recycling is crucial for foraging in complex environments. It leads to effective exploration of space for resources and exploitation of such substrate once located (foraging). As the resource level increases recycling becomes less significant: however recycling individuals are still marginally but not significantly fitter. From 5.5.3(b) (ii) the fungal individuals that are fit in 6 or more environments possess recycling capabilities. For two of the recycling individuals that are fit in all 10 environments their non-recycling counterparts are fit in 6 environments. These non-recyclers have high immobilization (non-insulated) and uptake rates and these traits are a better than average set of fitness attributes but an individual is fitter in more environments if in addition it possesses positive recycling attributes. Those non-recyclers that are fittest in a few environments are fittest in resource realisations that are not very patchy as in environments 2 and 7. Importantly, it is never a disadvantage to have positive recycling apparatus even in a homogeneous environment, assuming the metabolic cost associated with the process is not too costly. Positive recycling is clearly the optimal strategy in an unknown, patchy and resource limited heterogeneous environments such as soil.

This chapter demonstrates that a genotype encapsulating effective recycling promotes persistence in complex heterogeneous environments as it allows efficient discovery of resources. Further, these resources can be exploited fully due to the effective redistribution of biomass (both mobile and immobile), which is governed by the recycling process. However, in the soil environment fungi will often encounter other colonies and the interaction among these colonies has consequences on its fitness, functioning and the community composition. In the current formulation internal resource has been defined in a very generic sense to be that associated with biomass production only. However, internal resource involves a complex mix of components in real mycelia and each will be used for a range of purposes. These include the utilisation of resources in increasing bioavailability of external substrate, in signalling at the hyphal level, in reproduction, in producing inhibiting compounds during fungal interactions. The challenge of addressing biotic interactions that occur among fungal colonies is addressed in the chapter 6.

Chapter 6. Linking genotype to phenotype incorporating fungal interactions

6.1 Fungal Interactions: An Introduction

As colonies grow the network of hyphae expands and contracts and will almost certainly impinge upon the spatial territory of other mycelial networks, resulting in competition for space and resource over time. The outcome of such competition is a significant factor in shaping community structure. By considering, in the first instance, how mycelial distribution patterns emerge on agar, thus eliminating much of the complexity of a more realistic environment, the processes responsible for mycelial distribution patterns may be investigated. The challenge is to interpret these community-scale patterns in terms of the dynamic processes underlying the organisation of fungal communities and to transport this understanding into a more natural environment such as soil. To elicit the dynamical processes underlying a community the processes associated with interactions of individual mycelia must first be known.

There have been several experimental studies investigating the emergent mycelial distributions arising from confrontation between mycelia, usually paired oppositely on agar, e.g. Griffith *et al.* (1994); Boddy (2000); Rayner et al (1994); Stahl and Christensen (1992); White and Boddy (1992). The outcomes of interspecific fungal interactions can be categorised into competitive and neutralistic. Intermingling of hyphae, a neutralistic outcome, typically corresponds to the interaction of two fungi from the same isolate i.e. similar genetic material. However, intermingling of hyphae has occurred between two different isolates depending on the environmental conditions. The nutritional status of the environment can affect interactions outcomes. For example, low nutrition agar resulted in intermingling of different species, whereas highly nutritious agar resulted in deadlock of the same two species under identical conditions (Stahl and Christensen 1992). Other factors affecting interaction outcomes include water potential, temperature, inoculum size and stage of development (Boddy 2000, Stahl and Christensen 1992). Competitive outcomes occur either as a result of primary resource capture or combat (Boddy 1984). Competition as a result

ofprimary resource capture is dependent on colony extension and dispersal rates and the ability to utilise a resource. Combative interactions result in either replacement of one species by another or deadlock in which neither fungus enters the territory of the other. Deadlock interactions may be mediated at a distance through diffusible or volatile substances that result in inhibition or cessation of one or both mycelia, (Rayner and Webber 1984). Inhibition of one mycelium typically results in engulfment, and this may be a prelude to replacement. Replacement can occur as a result of direct interference of mycelia. Subsequently lysis can result, i.e. the destruction of the hyphal biomass as part of a metabolic process or as a reaction against detection of another mycelium. Lysis can result in resource being utilised biotrophically or necrotrophically by other fungi.

Although experiments on agar may provide us with knowledge of the range of possible interaction outcomes, the interpretation of outcomes obtained on agar cannot readily be extrapolated to a soil environment (Boddy 1984) in which other factors such as physical architecture of the soil and/or patchiness of resource may dominate the outcome. Falconer *et al.* (2005) showed that environmental context had a significant impact on colony morphology. Further, Falconer *et al.* 2006 (in preparation) demonstrates that different environmental contexts, e.g. level of nutrition in the environment, distribution of resources and structural distribution, affect the biological processes differently. For example, an individual possessing recycling apparatus growing in each of heterogeneous and homogeneous resource distributions produced different biomass distributions in each environment as a consequence of the recycling process and its response to that environment. It is envisaged that interaction processes will also respond differently to different environmental contexts impacting on the mycelial distribution

For example the physical architecture of soil governs growth and development of mycelial individuals (Ritz and Young 2004), and it is hypothesised that this has a significant effect on interaction outcomes. For a given three-dimensional volume of soil the degree of porosity and connectedness of the soil dictates, to a large extent, whether two mycelial colonies will compete. For low porosities there will exist unconnected paths and therefore fungi can coexist in isolation. However the more porous the soil becomes the more likely that competitive interactions will occur in order to secure space and subsequently any patchy resources.

This work extends the model to incorporate, for the first time, an interaction process that governs interaction outcomes based on associated trait values. The model is used to explore the effect of genotype and context on the phenotypes of interacting mycelia. In particular we address:

1. The effect of genotype on emergent mycelial distributions as a result of fungal interactions in a simple agar-like environment i.e. a homogeneous resource base;
2. the effect of resource context on interaction outcomes
    a. The effect of inoculum size on interaction outcomes
    b. The effect of external resource level on interaction outcomes;
3. the model is extended to three-dimensions and the effect of structural context, i.e. porous structure, on fungal interactions is explored.

6.2 Modelling Framework

The modelling framework extension takes the form of an inhibitor field that diffuses through the environment together with processes that determine colony response to that field. The colony response is realised by modifying the parameters of the existing model, and in particular the recycling and colony diffusion traits. The implementation is based on the following hypotheses relating to the interaction process.

1. **Inhibitor production:** It is known that the capacity to produce inhibiting compounds, extracellular enzymes and/or secondary metabolites is widespread in fungi (White and Boddy 1992). These compounds are derived from internal partitioning of resource within the colony and can either inhibit or kill competing fungi some distance away (Jennings and Lysek 1996). In agar, confrontation of two fungi paired opposite on agar plates usually results in mutual inhibition (Boddy 2000). This mutual inhibitor is attributed to a diffusible or volatile compound released by both colonies that prevents the local growth of the antagonist. In the model the rate of inhibitor production, i, is colony dependent and governed by a trait, π, which has a value

between 0 and 1. In the model there is no distinction between the different types of compounds that can be produced by the individual, e.g. inhibitor, antibiotic or extracellular enzyme and these are encapsulated in a single inhibitor field. This inhibitor field is derived from the mobile biomass concentration, n, and the conversion of mobile biomass into inhibitor has an associated metabolic cost, χ. The inhibitor field is diffusible with a constant diffusion coefficient, D_i, and, unlike the diffusion of mobile biomass is not constrained by the boundaries of the mycelium but by the limits of the environment.

2. **Inhibition:** Little is known of the mechanisms of inhibition (White and Boddy 1992) and in the model a global inhibitor field, i_g, which is the sum of all the inhibitor fields produced by individual colonies, i, in a given environment, exists. Locally, if $i_g > i$ this reflects the presence of another individual. A comparison of the genotype vector determines whether the individual is an antagonist or compatible i.e. different or identical genotypes respectively. If the inhibitor field is produced by an individual of the same genotype the two colonies will combine once their biomass fields connect, becoming one single individual. Otherwise, an antagonist is detected and the local diffusion coefficient of non-insulated biomass (D_b) is affected, preventing growth towards the antagonist. In the model implementation, growth is inhibited upon detection of an antagonist by assigning the local diffusion coefficient to zero. Each individual has a sensitivity threshold, ψ, as some colonies will be more sensitive to the inhibitor field than others. For an antagonist, if the global inhibitor field is greater than the colony inhibitor field by an amount greater than its sensitivity threshold i.e. ($i_g - i > \psi$) then the local diffusion coefficient for the non - insulated biomass is assigned zero, see e.g. 6.1. In all simulated experiments $\psi = 0.0$.

$$D_b = \begin{cases} D_b & i > i_g \\ 0 & i_g - i > \psi \end{cases} \qquad 6.1$$

3. **Lysis:** Lysis results in death of hyphal compartments followed by a programmed sequence of cytoplasmic destruction of one fungal colony usually upon contact of an antagonist (Boddy 2000). This can result in the antagonist overgrowing the lysised colony before the antagonist gains access to the resource. Lysis can be accompanied by antibiotic production (Lysek and

Jennings 1996). In the model, lysis occurs upon detection of an antagonist recognised by a non-self inhibitor field, i.e. ($i_g > i$), and the rate of lysis is controlled by trait, η. Lysis is simulated by changing the coefficients responsible for mobilisation and immobilisation of insulated and non-insualted biomass. If the colony has lysis capabilities, i.e. $\eta > 0.0$, and an antagonist is detected hyphal death and programmed cell destruction is simulated by a high mobilisation rate of insulated and non-insulated biomass, e.g. $\beta_l = \beta_n = 0.9$. This converts hyphal biomass into mobile phase. Correspondingly there is a low rate of immobilisation of both insulated and non-insulated biomass, e.g. $\alpha_l = \alpha_n = 0.0$, reducing biomass assimilation, see e.g. 6.2.

$$\alpha_i = \begin{cases} \alpha_i & i > i_g \\ 0 & i_g - i > \psi,\ \eta > 0 \end{cases} \qquad \beta_i = \begin{cases} \beta_i & i > i_g \\ 0.9 & i_g - i > \psi,\ \eta > 0 \end{cases}$$

$$\alpha_n = \begin{cases} \alpha_n & i > i_g \\ 0 & i_g - i > \psi,\ \eta > 0 \end{cases} \qquad \beta_n = \begin{cases} \beta_n & i > i_g \\ 0.9 & i_g - i > \psi,\ \eta > 0 \end{cases} \tag{6.2}$$

Once the total immobile biomass ($b_i + b_n$) is much smaller than mobile biomass (n) i.e. ($b_i + b_n < 1^{-10}$ and $n > 0.0$) then the mobile biomass is converted into environmental resource, this reflects rupturing of cell membranes and loss of cytoplasm. The resource released from lysis can therefore be utilised necrotrophically by other colonies.

The set of physiological processes including the interaction process results in a vector of 12 parameters: α_n, α_i, β_n, β_i, θ, λ_1, λ_2, ξ, D_n, D_b, π and η (red highlights the extension traits and equations). The resultant set of equations describing uptake, biomass production and recycling, the transport of mobile biomass and interactions amongst mycelia may be written as:

$$\frac{\partial b_i}{\partial t} = \zeta\left[\frac{\partial}{\partial x} D_b(i)\frac{\partial b_n}{\partial x} + \gamma\left(\alpha_n \pi^\theta - \beta_n \pi\right) b_n\right] + \gamma\left(\alpha_i \pi^\theta - \beta_i \pi\right) b_i,$$

$$\frac{\partial b_n}{\partial t} = (1-\zeta)\left[\frac{\partial}{\partial x} D_b(i)\frac{\partial b_n}{\partial x} + \gamma\left(\alpha_n \pi^\theta - \beta_n \pi\right) b_n\right],$$

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} D_n(n)\frac{\partial n}{\partial x} - \left(\alpha_n \pi^\theta - \beta_n \pi\right) b_n - \left(\alpha_i \pi^\theta - \beta_i \pi\right) b_i + \left(\lambda_1 b_n + \lambda_2 b_i\right) s - \eta n,$$

$$\frac{\partial s}{\partial t} = \omega(s_m - s) - \left(\lambda_1 b_n + \lambda_2 b_i\right) s + \eta n,$$

$$\frac{\partial i}{\partial t} = \pi \chi n + \frac{\partial}{\partial x} D_i \frac{\partial i}{\partial x}$$

The system of equations was discretized on a 2D square lattice large enough to visualise the colony morphologies of two species. In Chapter 4 the modelling of single colonies was based on a square grid size of 128. For some consistency in space, for two species interactions a square lattice of 256 x 256 is used. For three-dimensions a 48 x 48 x 48 cube was used as this was achievable computationally. Both two and three-dimensions were solved using the Crank Nicholson implicit method in conjunction with successive over relaxation. No flux boundaries were imposed.

6.3 Scenarios

6.3.1. The effect of genotype on emergent mycelial distributions

To determine the impact of genotype on emerged mycelial distributions a consistent and simple environment is used. The environment is 2-dimensional and homogeneous with respect to resource, 1 unit per cell, and structure reflecting an agar system. Simulated experiments included mostly confrontation between two different species i.e. two different genotypes. However in one case the interaction between two identical species is investigated. The non-interaction trait values of the fungal individual were based on the fittest individual identified in Chapter 5. For simulations based on two species, the two colonies differ only by their recycling traits ((α_n = 0.87, α_i = 0.0, $\beta_n = 0.0$, $\beta_i = 0.0$) and (α_n = 0.87, α_i = 0.01, $\beta_n = 0.0$, β_i = 0.34)) to determine the effect of the traits associated with the interaction process (π and η) on emergent mycelial structure. For the simulation investigating the interaction between two colonies of the same genotype the recycling

genotype detailed above was used i.e. (α_n = 0.87, α_i = 0.01, $\beta_n = 0.0$, β_i = 0.34)) to determine the effect of the traits associated with the interaction process (π and η) on emergent mycelial structure.

6.3.2. The impact of resource context on mycelial distributions

The mycelial distributions observed in the field are sensitively dependent on context, be it level of external resource or innoculum size (Boddy 2000). In order to determine the effect of resource context on simulated mycelial forms two scenarios are investigated.

6.3.2.1 The effect of resource level

The simulated environment is the same as in scenario 1 i.e. 2-dimensional and homogeneous with respect to resource and structure. However the level of resource in each cell of the 2-dimensional environment is set to 1.0 or 0.0001 units per cell. The two species of Scenario 1, i.e the recycling and non-recycling individuals, were used to investigate the effect of resource level on mycelial distributions.

6.3.2.2 The effect of inoculum size

The effect of inoculum size was investigated using two individuals with different genotypes. The genotypes of both individuals were varied, starting from the genotypes used in scenario 1, in order to determine if a different interaction outcome can be obtained as a result of changing the inoculum size.

6.3.3 The impact of structural context on mycelial distributions

Results of interaction outcomes on agar may not inform us of what is happening in the natural environment (Stahl and Christensen 1992) and so the use of a more realistic environment may aid our understanding as to how physical architecture affects emerged mycelial distribution of two interacting colonies. Knowledge of how structure affects interactions is essential to understanding the community structure and functions of fungal communities in soil. These colonies have the same trait sets as in Scenario 1 and so deadlock is the inferred interaction outcome from the results of Scenario 1. These two individuals are inoculated at opposite planes of a 48*48*48 volume. The

volume has an associated pore space distribution reflecting the bulk porosity of the volume. Each voxel has a probability 1-p that it is a pore, and p is varied between 0 and 1. For a given bulk porosity measure, 120 different realisations are obtained using a different seed for the random number generator. The two fungal colonies are subsequently grown through these 120 structure sets.

6.4 Results

6.4.1 Impact of genotype on agar interaction outcomes

For a homogeneous environment the model replicates interaction outcomes observed on agar i.e. deadlock, engulfment, replacement and intermingling. By varying the mycelial genotypes that govern the growth of, and interactions among, individuals a range of mycelial distributions emerge as a consequence of the interactions between the genotypes and the environment.

6.4.1.1 Deadlock

The colonies of Figure 6.1a) have similar genotypes apart from recycling mechanisms but both have inhibitor releasing qualities ($\pi > 0$). Both colonies produce an inhibitor field and in both cases the detection of an inhibitor field that is non-self ceases local growth. This interaction stops growth of both mycelia in the direction of each other well before any contact is made resulting in deadlock Fig. 6.1(b)

6.4.1.2 Replacement – Engulfment

Engulfment of one colony by another can occur when only one colony possesses inhibitor-producing capabilities ($\pi > 0$). From Fig. 6.2(a) there are initially two colonies with similar trait sets but colony A (left hand side) does not release an inhibitor whilst colony B (right hand side) does. The effect of colony B producing an inhibitor field affects the growth of colony A by ceasing its growth. Since colony A does not produce an inhibitor, colony B continues to grow engulfing colony A as illustrated in Fig. 6.2 (b). Engulfment will eventually lead to replacement of colony A as over time it will become insulated and decay will ensue.

6.4.1.3 Replacement – Lysis

Fig. 6.3(a) shows the relative proportions of two inocula corresponding to two different mycelial genotypes. The smaller inoculum, colony A, has inhibitor producing capabilities and no lysis mechanism ($\pi > 0$, $\eta = 0$) whilst the more substantial innoculum, colony B, has lysis capabilities but no inhibitor producing mechanism ($\pi = 0$, $\eta = 1$). Due to colony A's release of inhibitor, colony B stops growing and since it has lysis properties the immobile biomass is converted into mobilised at a high rate which is subsequently released into the environment. This release occurs when the mobile biomass is greater than zero and the immobile biomass is low ($< 10^{-10}$). This sequestered resource can ultimately be utilised by another organism such as colony A, the peak of biomass in Fig. 6.3b corresponds to the resources gained by lysis of colony B.

6.4.1.4 Intermingling

In Fig. 6.4(a) the genotypes of the two colonies are identical, i.e. they possess recycling apparatus and inhibitor producing capabilities ($\pi > 0$). For two colonies releasing inhibitor, deadlock, as in Fig. 6.1b, would normally result but since these colonies are identical genetically intermingling occurs, Fig. 6.4(b).

6.4.2 Impact of context on interaction outcomes

6.4.2.1 Effect of context - external resource

In Figure 6. 5(a) the amount of resource in each cell is low (0.0001) leading to intermingling of fungal biomass even though $\pi > 0$. In Fig. 6.5b) the amount of resource in each cell is high (1.0) leading to deadlock. When the resource level is low there will be little investment in an inhibitor field therefore its presence will take a longer time to detect or may not be detected at all as it is so low. Once the resource level is increased then the inhibitor field increases in magnitude and can be detected.

6.4.2.2 Effect of context - inoculum size

If the inoculum size of the two colonies is equal as in Figure 6.6(a) then the interaction outcome resembles deadlock. The morphology of the emerged mycelial deadlock is different from the deadlock of scenario 1 as the colonies have different growth and insulating rates. Colony A has a higher extension rate so grows faster and occupies more space. However, if the inoculum size of colony A is 10 times bigger than colony B, then B's growth is inhibited sooner as there is more investment in the inhibitor field by A. Therefore its presence is detected sooner by B for the same sensitivity threshold. This leads to partial engulfment of B by A as in Fig. 6.6 (b). By changing the inoculum size, the emergent mycelial distribution changes from a deadlock interaction to a partial engulfment outcome.

6.4.3. Effect of context - porous structure on deadlock interaction outcomes

The probability (out of 120 realisations) that each colony A and B and both colonies A and B grow to the opposite plane from the point of inoculation is recorded and is shown in the graph of Fig. 6.7. The porosity window in which both fungi can percolate to the opposite plane of a 3D volume is limited (0.315 – 0.53). At low porosities (< 0.315) percolation of both fungi through the structure is rare due to limited connected pathways connecting the opposite planes. At intermediate porosities there exist several connected paths, and not all of which have been secured by the opposing fungus, and so both fungi can grow past one another (deadlock preventing intermingling of connected paths). At high porosities (> 0.53) percolation of both fungi is unlikely, as the opposing fungus has secured most of the spatial territory at the opposite plane resulting in complete deadlock.

The probability that two fungi grow through the entire structure is reminiscent of percolation theory and it has been shown for a single individual in 2-dimensions that fungal invasion (percolation) can be stopped by a threshold population of randomly removed resource sites (Stacey *et al.* 2001). If the probability that both colonies reach the opposite plane is a percolation system then the trend

line characterising the probability of percolation will follow a power law near critical points, i.e. critical threshold. Figure 6.8 shows the first 10 points after the critical threshold (0.315) and the corresponding power and linear functions describing the data points. The power law fits the data points best with a regression coefficient of $R^2 = 0.9911$ which is significantly better than the linear fit suggesting a behaviour characteristic of a percolation system.

6.5 Discussion

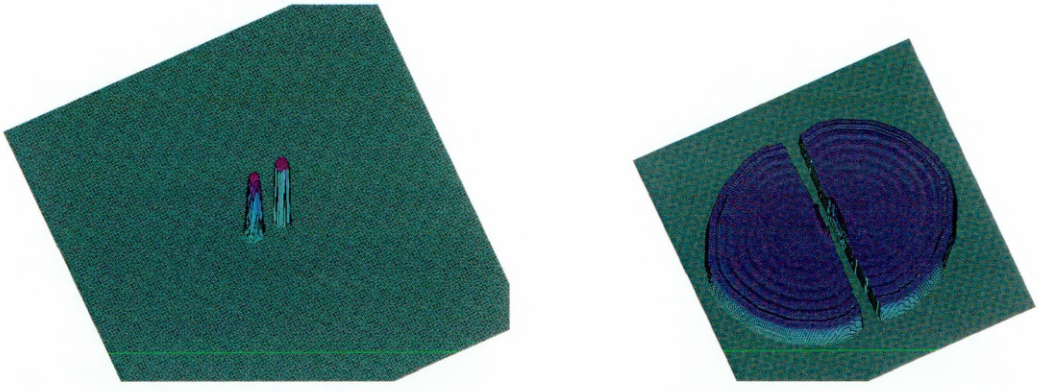
The extended model incorporates processes responsible for emergent mycelial distributions as a result of both colony growth and interactions. Observed mycelial distributions are reproduced and may be interpreted as resulting from relatively simple interaction between localized processes governing recycling of mobile biomass and colony scale transport within a mycelium and interactions among mycelia. An additional process that allows detection of other mycelia mediated via the environment is used to describe these interactions. Broad ranges of observed mycelial distributions e.g. deadlock, intermingling, engulfment and replacement arise from different realisations of these processes as characterised by different trait values and from different environmental contexts. Further, resource level plays a critical role in determining interaction outcomes where deadlock is the outcome if resource level is high and intermingling if resource level is low (Figs 6.5 (a) and (b)). This is consistent with experimental results in Stahl and Christensen (1992) where the outcome of the interaction between *Penicillium restrictum* and *Humicola fuscoatra* was investigated on agar with different nutritional status. On malt agar (high resource base) the interaction outcome was deadlock, where colony growth towards one another completely stopped at a separation distance of > 1.0cm. In contrast the interaction outcome was neutral intermingling on plate cultures containing dilute cornmeal agar (low resource base). The model shows how environmentally mediated cues are involved in emergent mycelial distributions and how sensitive the interaction outcomes are to environmental and physical context.

As well as environmentally mediated cues and resource context, the physical architecture of the habitat plays a significant role in determining mycelial distributions. In particular for soil environments the pore geometry will to a large degree dictate the interaction outcome. The percolation plot, Fig. 6.7, demonstrates that the percolation of two fungal colonies, in a 3-

dimensional porous structure, to the opposite plane from the point of inoculation undergo a continuous phase transition. At a critical threshold (0.315), a spanning cluster of pores exists that connects through the 3D lattice. This causes a phase transition from cessation of growth of both colonies to the percolation of both mycelia through the volume. This critical value is consistent with other 3D percolation systems (Stauffer and Pandey 1992). The power law shape of the percolation plot after the critical threshold (Fig. 6.8) indicates that the system is a percolation system.

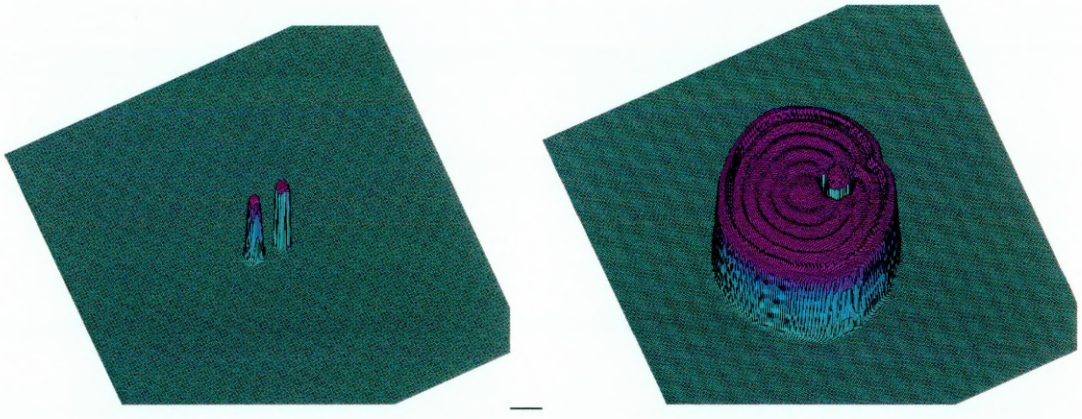
This formulism demonstrates that the phenotype of interacting mycelia results from simple local rules spatially mediated by external transport of an inhibitor. The model can now be used to investigate how individual-scale behaviour and emergent community-scale functioning may be understood in terms of relationships among traits, i.e. fungal ecology. Specifically, it can be utilised to identify which trait values of fungal colonies (if any) allow coexistence in a given environment and the effect of heterogeneous structure and resource on the form of coexistence.

Figures



(a) t = 1 (b) t = 13

Figure 6. 1Deadlock resulting from both fungal individuals producing inhibitor and their growth being sensitive to the presence of the other. The trait values are [α_n = 0.87, α_i = 0.0, β_n = 0.0, β_i =0.0, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.01, η =0.0] and [α_n = 0.87, α_i = 0.01, β_n = 0.0, β_i =0.34, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.00, η =0.0]



(a) t = 1 (b) t = 13

Figure 6. 2 Engulfment of colony A (left hand side) by colony B (right hand side). The trait sets for colony A and B are [α_n = 0.87, α_i = 0.0, β_n = 0.0, β_i =0.0, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.0, η =0.0] and [α_n = 0.87, α_i = 0.01, β_n = 0.0, β_i =0.34, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.01, η =0.0]

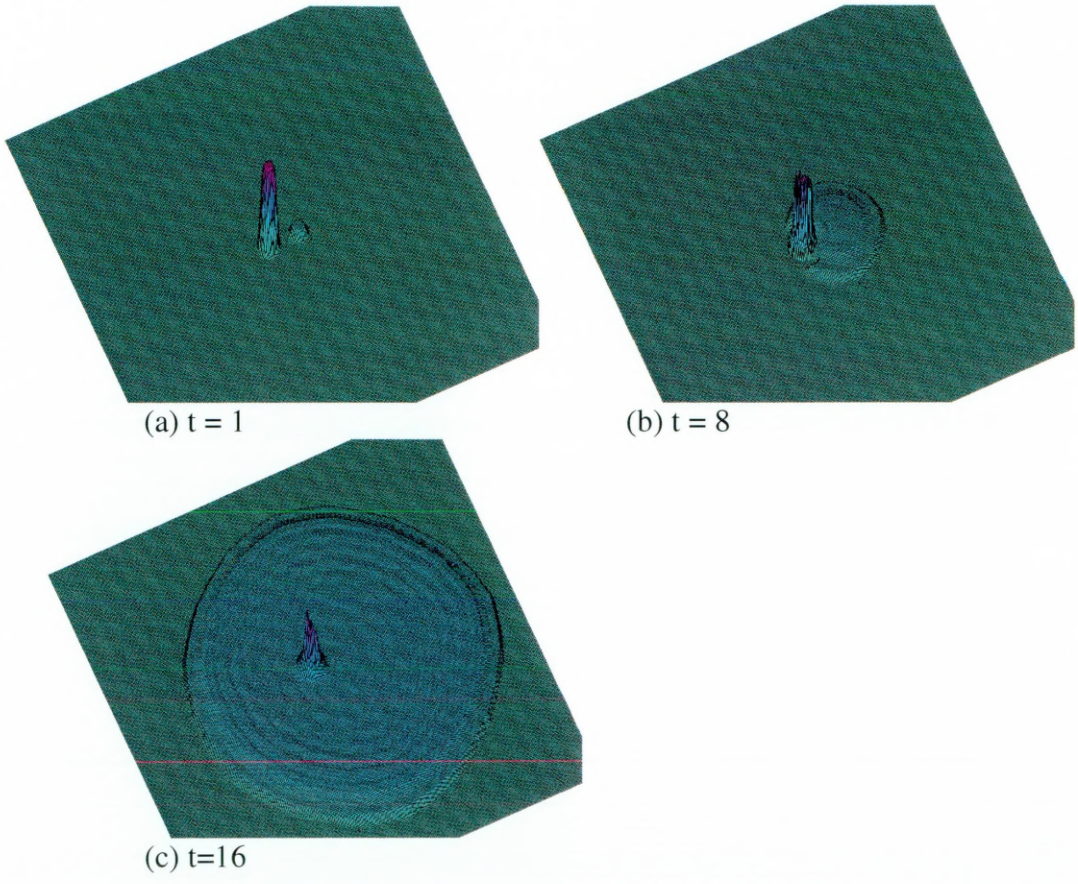


Figure 6. 3 Replacement of one fungus by the other. In (a) the smaller inoculum (colony A) replaces the larger inoculum (colony B) , (b) colony A engulfs colony B and (c) colony A replaces B.The corresponding trait sets of and [α_n = 0.87, α_i = 0.0, β_n = 0.0, β_i =0.00, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.01, η =0.0] and [α_n = 0.87, α_i = 0.01, β_n = 0.0, β_i =0.6, θ =1.0, λ_1 =0.65, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.0, η =1.0]

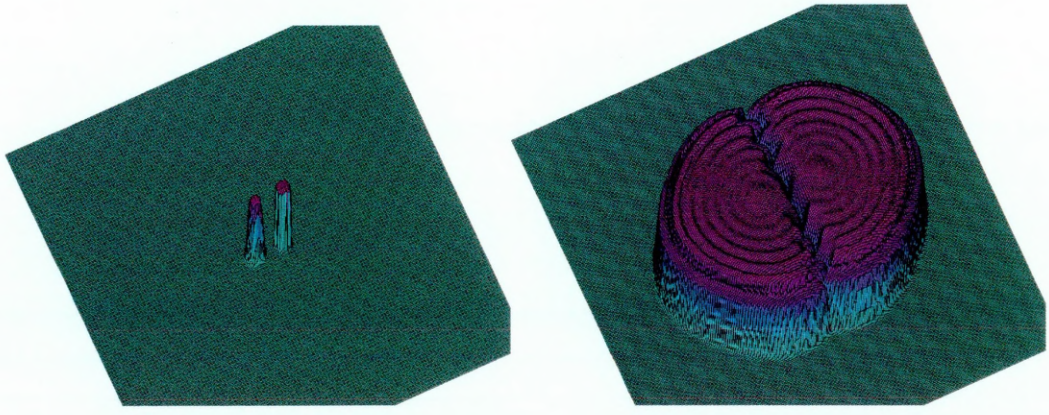
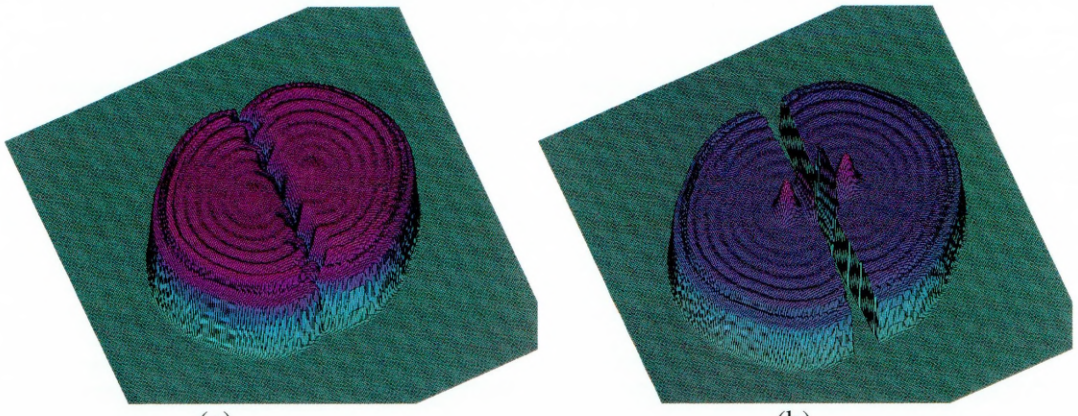
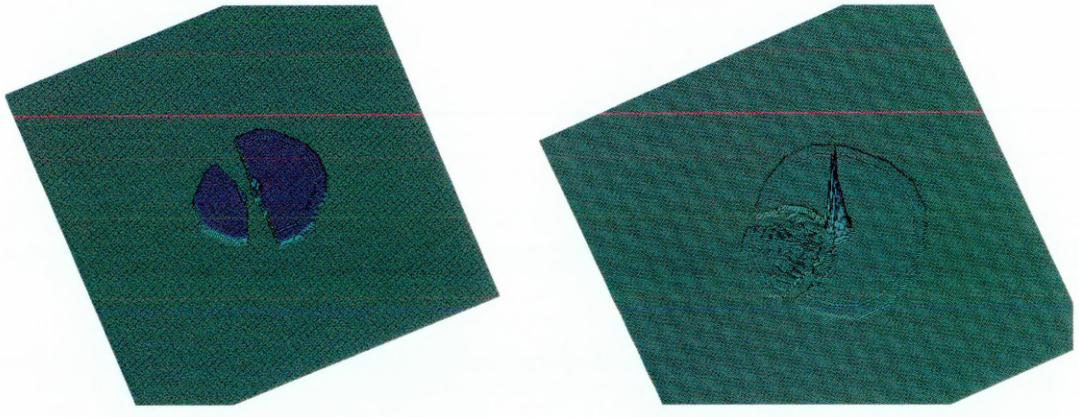


Figure 6. 4 Trait sets of colony A and B are identical and are same as colony A in Fig. 1[α_n = 0.87, α_i = 0.0, β_n = 0.0, β_i =0.0, θ =1.0, λ_1 =0.97, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.01, η =0.0]. Since the genotypes are the same intermingling occurs.



(a) (b)

Figure 6. 5 Colonies have same trait sets as in Fig. 6.1 (a) low external resource, intermingling (b) high external resource, deadlock



(a) **(b)**

Figure 6. 6 Colonies have trait sets of : [α_n = 0.87, α_i = 0.01, β_n = 0.6, β_i =0.34, θ =1.0, λ_1=0.95, λ_2 = 0.1, ξ = 0.05, $D_n = D_b$ = 5.0, π =0.01, η =0.0] and [α_n = 0.65, α_i = 0.01, β_n = 0.4, β_i =0.6, θ =1.0, λ_1=0.95, λ_2 = 0.1, ξ = 0.01, $D_n = D_b$ = 10.0, π =0.01, η =0.0] respectively. In Fig. (a) the inocula of colony A and colony B are equal. In Fig. (b) the inoculum of colony A is 10 times greater than that of colony B.

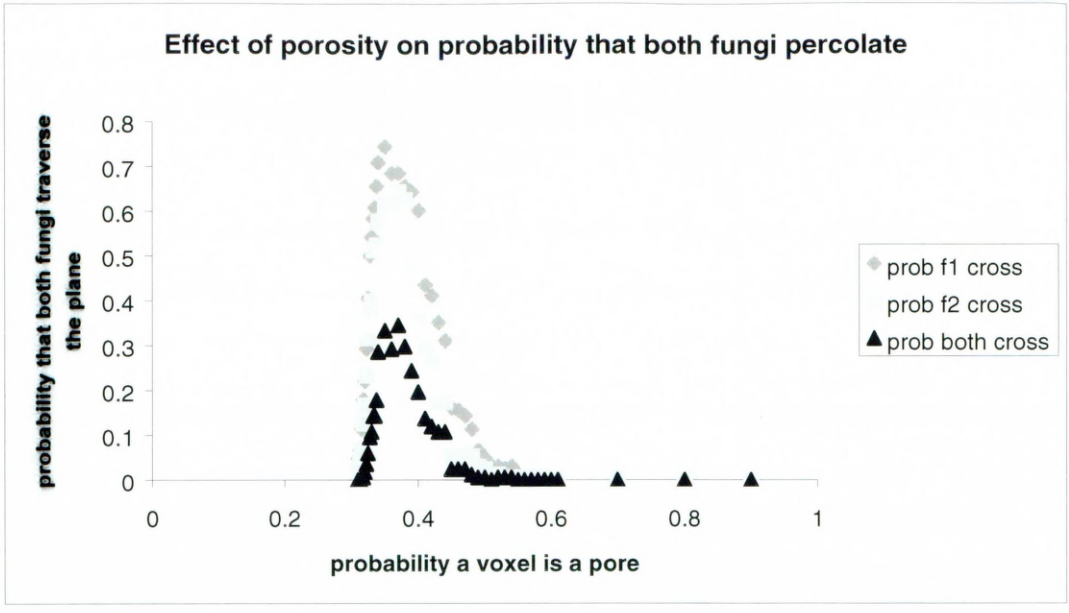


Figure 6. 7 Probability of percolation of each and both colonies

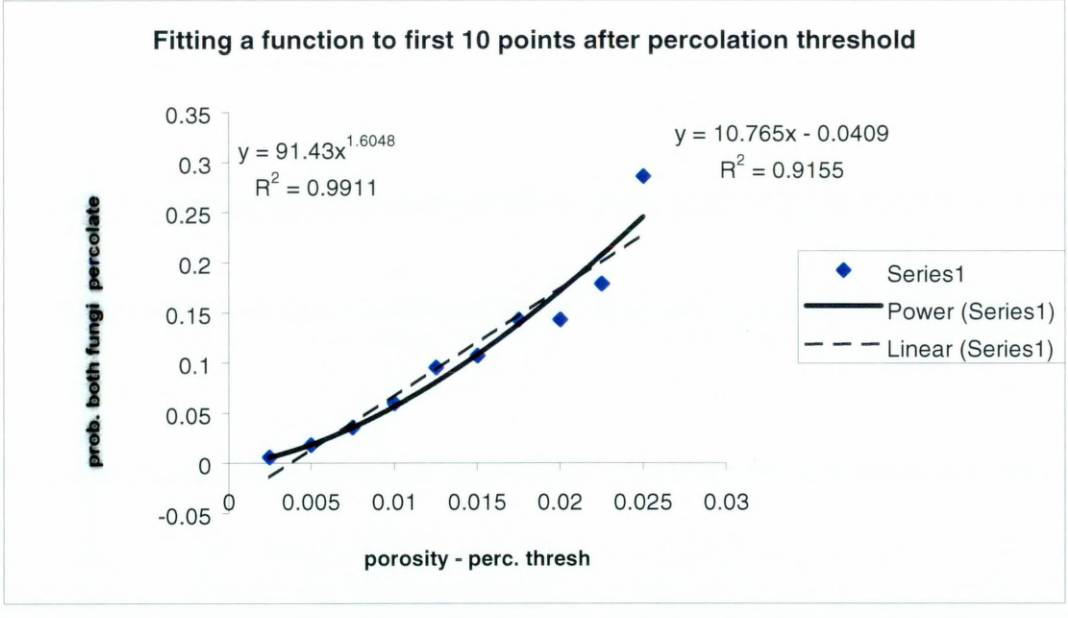


Figure 6. 8 Mapping of power and linear function to 10 points after the percolation threshold.

Chapter 7. Associated and Future work

Opportunities have arisen as a result of the work undertaken as part of this doctoral program that allows a number of associated and future projects. This involves extensions to both the single and multiple colony models and will be discussed below.

7.1 Associated work with regard to the single colony model – The discrete hyphal model

The model presented in Chapter 3 describes how hyphal densities change locally as a response to the interaction between environment and genotype. The unit of this model is a biomass density measure as it was the intention to scale up to investigate interacting mycelia and communities as discussed above. The density model assumes isotropic connectivity of hyphae and therefore complete mixing of the mobile biomass locally. This assumption is somewhat unrealistic and we need to ensure that the patterns obtained are not a consequence of this local averaging. We can investigate how stable these patterns are to the occurrence of discrete hyphae. In natural soil environments the distribution of pore sizes is enormous ranging from nanometres to centimetres. It would be of interest to investigate the factors influencing hyphal penetration of the environment at smaller scales. There have been some experimental studies in this area, e.g. Money (2004), and the direction of growth of individual hypha is influenced by the texture of the environment. It has been found that hyphae prefer to grow along the surface of a macropore rather than bridge an air gap, this is most likely due to the energy costs associated with bridging a gap (Ritz and Young 2004) or more likely to come into contact with resource if they remain in contact with the substratum. Further, hyphal densities in soil vary considerably but away from resource the hyphae grow sparsely in the soil environment, i.e. it is often possible to distinguish individual hypha at the pore scale. As far as we are aware texture is not a feature of any existing hyphally discrete model even though it plays a major role in the growth direction of hyphae. The current colony scale model has been discretized in terms of hyphae, within the context of a related doctoral program of study, so that hyphal mapping of the colony may be determined and tracked as a response to the environment. This hyphal-scale model is based on the same physiological processes assumed in

the biomass density model presented in Chapter 3 and the hyphally discrete versions of the phenotypes can be obtained (e.g. Fig. 7.1). In the discrete model the uptake, recycling and redistribution processes are the same as in the hyphal density model. The growth process however is not mediated by diffusion but is based on a probability that the hyphae will grow randomly into one of six orientations, once it exceeds a biomass threshold i.e. Brownian motion. Growth of hyphae occurs on a two-dimensional hexagonal lattice. Biomass heterogeneities in the discrete profiles did not appear until the nonlinearity associated with the recycling was greater than one. This was true of the density model also. Fig. 7.1 shows a hyphally discrete simulated colony growth form on a two-dimensional agar-like environment, replicating the concentric rings of Fig. 4.1(e) of Chapter 4. Therefore the mean field model results are not an artefact of spatial averaging. However, the rings disappear when anastomoses is precluded therefore some degree of connectedness is required. The growth process can now be extended to incorporate the effect of texture at the pore scale on the growth orientation of hyphae.

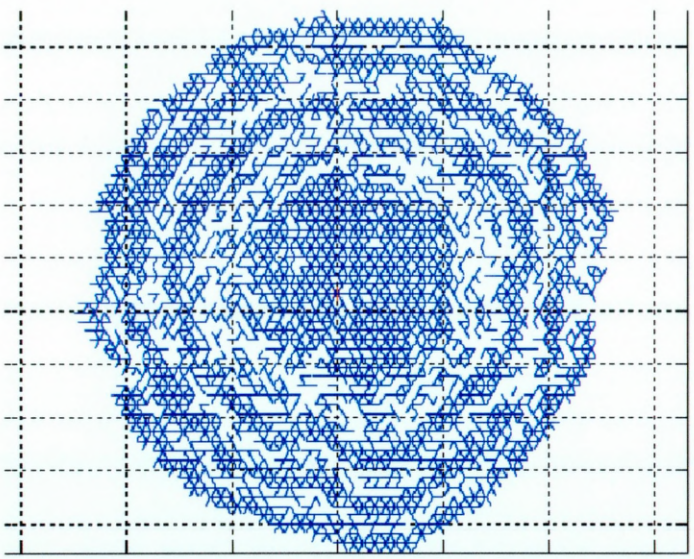


Figure 7. 1 2D hyphally discrete biomass profile of figure, concentric rings of high and low hyphal densities appear once the nonlinearity associated with biomass recycling is greater than one.

7.2 Future work with regard to the single colony model - Complimentary qualitative mathematical analysis

For a more complete analysis of the reaction diffusion system (box 3.1) the author wishes to carry out some complimentary qualitative mathematical analysis on the equation set. The numerical analysis has indeed been useful in identifying the form of the solution for specific initial and

boundary conditions. The identification of generic behaviour such as considering the stability of the uniform steady state solutions and the existence of travelling waves would add to the information already known regarding the system.

For the linear stability analysis this would involve the identification of non-negative equilibrium points (as the colony cannot have a negative density measure), followed by sensitivity of stability to a change in parameter value i.e. the steady state will then be subjected to small perturbations to ascertain if it is stable. An equilibrium solution of a reaction diffusion problem is stable if all time-dependent solutions starting out sufficiently close to the equilibrium stay in any given neighbourhood of it for all t >0 (Grindrod 1991). An equilibrium or steady state is unstable if one can find solutions starting arbitrary close to it, that leave some given small neighbourhood. An investigation into the existence of travelling waves of the reaction diffusion system (box 3.1) would determine the parameter values that permit the advancement of fairy rings – a natural form of travelling wave. From this it is possible to determine the speed of the travelling wave and validate this by rate of advancement of fairy rings in nature. Fig. 3.1 qualitatively shows a travelling wave. However the mathematical analysis has not been undertaken regarding the speed and type of travelling wave.

7.3 Associated work regarding the fungal interaction model - Origin of diversity in fungal communities

The model can be used to address issues relating to diversity of fungal communities. Ideally this should be undertaken using a parameterized and validated model as described in section 7.4. Here, a theoretical preliminary investigation into fungal diversity is underway. There can be up to 10^6 propagules of fungi in 1 gram of soil yet knowledge of mechanisms promoting diversity is scant (Deacon 2005). Spatial and temporal heterogeneity are the key external environmental factors that promote coexistence of species (Shea and Chesson 2000). Ecologists have also noted that the cost of performing one ecological function well (e.g. consuming one type of resource) comes at a cost of performing another function (e.g. consuming another type of resource), (Kneitel and Chase 2004). This is hypothesised to occur due to physical/physiological trade-offs between traits of organisms. This prevents a Hutchinsonian demon whereby one species in a community dominates because it is the best at everything, e.g. colonising new patches, utilising all resources, avoiding

predators and resisting stresses, often referred to as a superorganism (Kneitel and Chase 2004). The theoretical model can be used to explore the possibility of interspecific trade-offs among properties of interacting species, spatio-temporal heterogeneity and disturbance on fungal diversity. A preliminary study investigating the role of interspecific trade-offs among species is underway. An unstructured three-dimensional environment is randomly seeded with 120 randomly generated genotypes. These genotypes control the rate of uptake, recycling, redistribution, growth and interactions among colonies. The number of coexisting genotypes is monitored through time, (Fig. 7.2). The figure shows a rapid decline in number of species (diversity) during the initial phases of the simulation, and settles to a steady number of individuals. The biomass distribution of coexisting types was plotted for the last generation (Fig. 7. 3) and this follows a lognormal distribution (Fig. 7.4). This relative abundance distribution describes how the individuals in a community are partitioned among rare and common species. The lognormal form of the distribution indicates that the community contains many rare species and only a few common ones, and is associated with a community in equilibrium (Pachepsky 2001). The factors promoting the coexistence of these 42 individuals, e.g. does an interspecific trade off exist, is currently being investigated. This investigation can be extended to study the impact of spatial heterogeneity in the form of physical architecture and/or environmental resource on diversity.

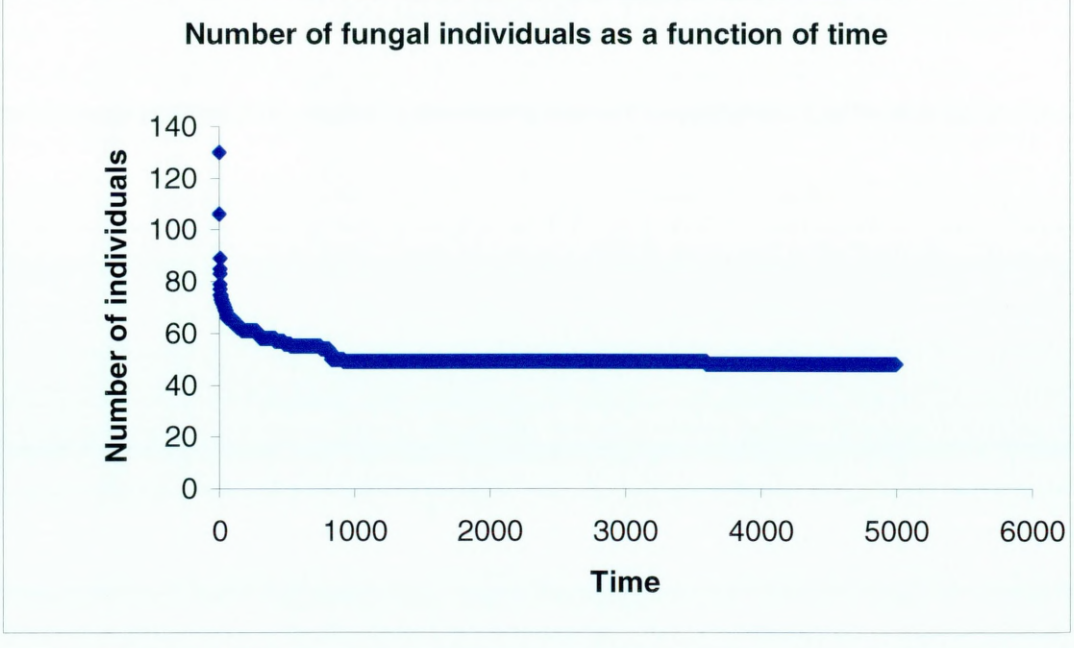


Figure 7. 2 The number of fungal individuals as a function of time. Diversity drops and quickly settles to a steady number of coexisting fungal individuals

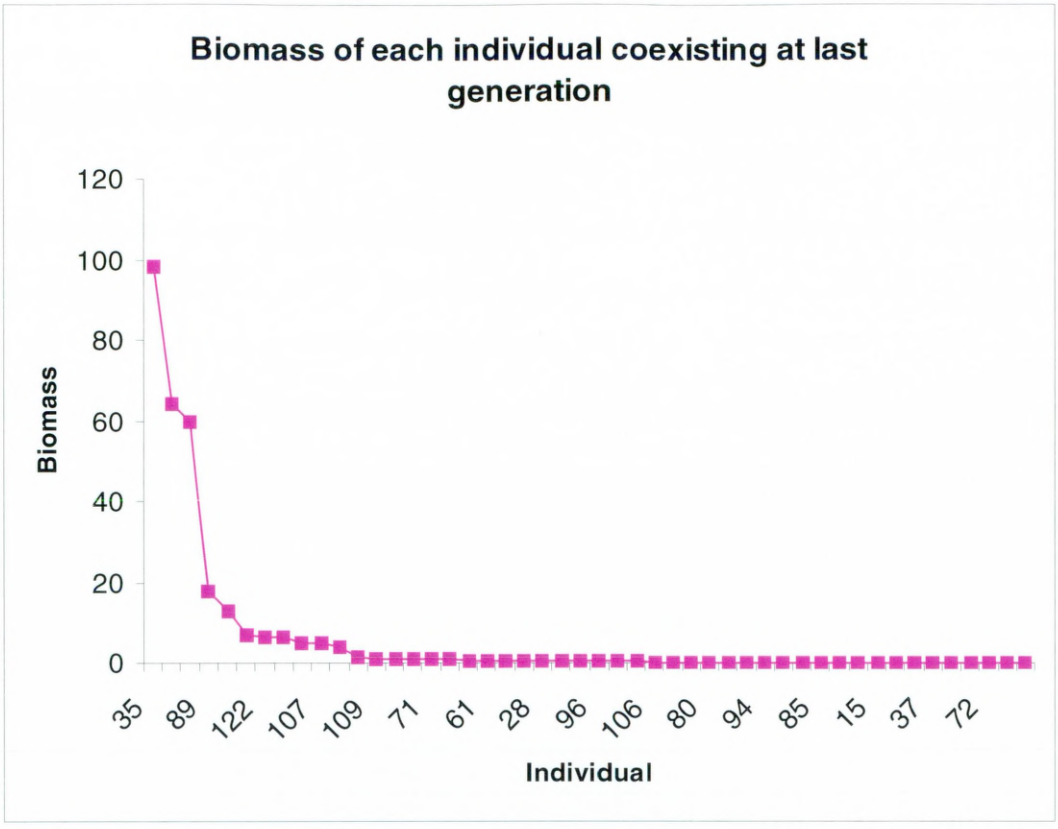


Figure 7. 3 Biomass (abundance) distribution of the coexisting individuals at generation 5000

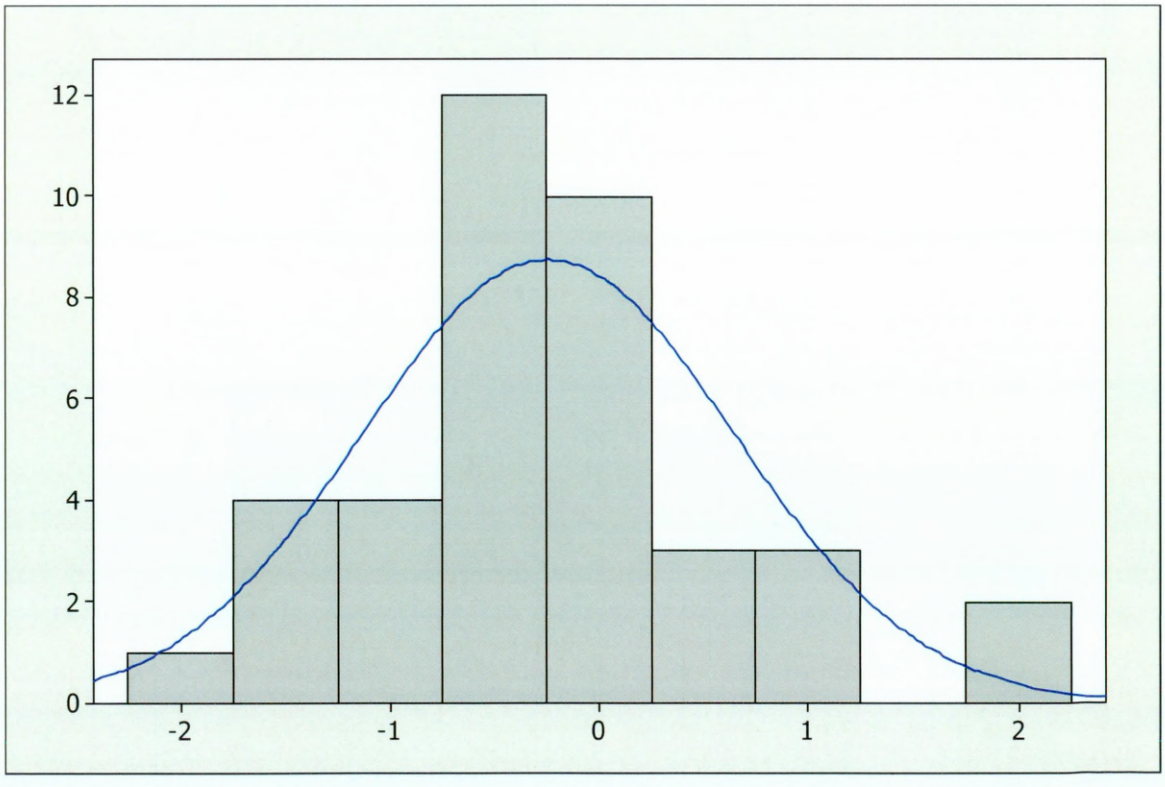


Figure 7. 4 Lognormal distribution of coexisting biomass abundances. Obtained by taking the log of the biomass abundances and placing them in a histogram.

7.4 Future work regarding the fungal interaction model – Parameterisation and validation of the theoretical model – towards a predictive model

An experimental system developed by RISO National Lab, Copenhagen provides a unique way of determining the impact of spatial heterogeneity on the growth form of fungal colonies. The system may also be capable of determining the effect of chemical and biotic heterogeneities on fungal form.

Experimental System

Riso have developed a unique and exploitable process to create a silicon-based 'glass soil'. The process utilises industrially relevant polymer fabrication methods and is able to create transparent complex spatial structures at the nano and micro-scale. Moreover the fabrication process allows prescription of that spatial structure from image maps including soil thin sections as in Feeney *et al.* (2006), Fig. 7. 5. The maximum physical dimensions of the fabricated structures are a few centimetres in the horizontal plane. Further, a small number of these structures may be stacked on top of each other, offering a limited three-dimensional environment, Fig. 7.6. The underlying physical structure can be modified to replicate 7. 5, a real two-dimensional thin slice of soil, and a few of these can be stacked in three dimensions as in Fig. 7.6. A fungal colony can be grown through the limited three dimensional environment, which mimics the physical architecture of a soil environment. The hyphae will grow where there is a pore and be constrained where there is silicone. Riso have demonstrated proof-of-concept where fungal hyphae are profiled growing through a regular physical glass-soil structure (Fig. 7. 7). The hyphae grow avoiding the square columns and grow through the pore space.

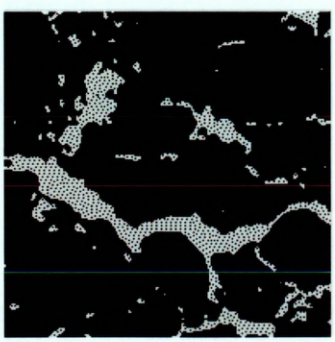


Figure 7. 5 2-dimensional soil thin slice obtained from Computed Tomography of a 3-dimensional core of field soil. Grey represents pore space and the back solid phases.

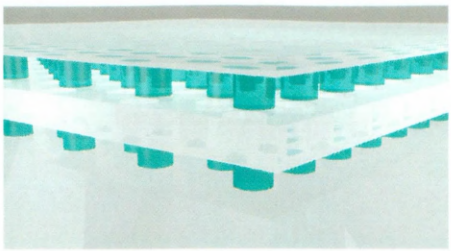


Figure 7. 6 stacking of the 2D slices, in the illustration above the columns will represent solid phases and will be arranged more irregularly when a number of 2-dimensional silicone slices based on real soil thin sections are stacked.

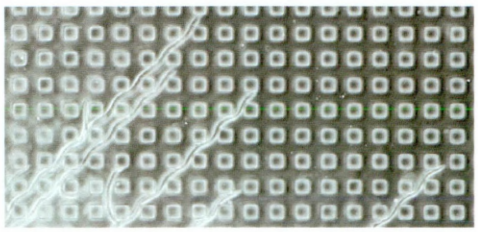


Figure 7. 7 fungal hyphae growing through a single slice of a regularly structured silicone slice. The silicone structure produced here was not based on a soil thin section

Experiments are initiated by placing structured growth media next to the leading edge of growing mycelium on an agar plate. Funnel-like features of the silicone structured growth media force the hyphae to enter the channel structures of the medium (sketch of the approach of the preliminary experiments below Fig. 7.8). The interior of the 3D structure is made hydrophilic so that hyphae of *Rhizoctonia solani* can grow through hydrated pores of a single-layer structure (Fig. 7.7).

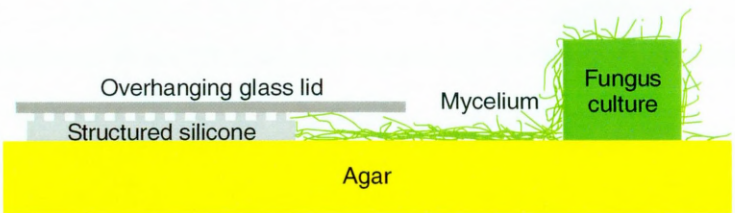


Figure 7. 8 Experimental set up illustrating how fungi are channelled into the silicone environment

Growth and morphogenesis of the fungi can be studied and documented by time-lapse light microscopy. Suitable microscope set-ups as well as tracking and morphology software have already been developed for this purpose. Confocal microscopy using reflected light either with or without a Green Fluorescent Protein (GFP) expressing mycelium will be used for detailed studies of the hyphal growth patterns in the multi-layered structures. Important, novel aspects of these investigations are the abilities to compare growth in pore systems of different dimensions and of

different chemical patterning, and to observe growth *in situ* over time. Such studies have previously not been possible and will provide experimental foundation for modeling the fundamental growth strategies and responses of fungi.

The Riso experimental system can be combined with the theoretical model and Computed Tomography scanner facilities at UAD to inform us of the dynamics of fungal colonies growing in a natural soil environment. First, experimental systems may allow measurements of uptake, growth, recycling, internal transport, and rate of insulation traits in a wide range of contexts. They may also provide us with the 3D profiles of mycelium that can subsequently be attributed a space filling metric such as fractal dimension that may then be compared. The three-dimensional profile of fungal hyphae using CT scanner may be possible by loading the mycelium with heavy metals and using sophisticated segmentation techniques. Using carefully designed experiments, upper and lower bounds for each colony specific trait can be obtained. For each parameter the experiments would be constructed in order to elicit this information. For example two silicone structures both with the same bulk density but low and high pore tortuousness may allow the identification of upper and lower bounds for the diffusion coefficient, which is related to the extension rate. It is envisaged that immobilisation and mobilization i.e. recycling can be derived from some form of quantification of net local change in biomass density and insulating capabilities can be informed by the degree of anastomoses.

The parameterised theoretical model can be used to bridge the gap between a practical experimental system and the real system. This typically involves a number of steps:

1. An experimental set up with an additional complexity e.g. topology, chemical or microbial heterogeneity is compared against a replicated theoretical situation using the parameterized model and the observed and theoretical results are compared. A search of trait space will identify the parameter set with the 'best fit' between simulated and observed behaviour.
2. It is likely that there will remain differences between simulated and observed outcomes due to the additional added complexity e.g. topology, chemical and microbial heterogeneity that

was not incorporated in the initial parameterisation. To overcome this difference a correction factor can be added.

3. The correction factor is an additional parameter that will alter the theoretical model's output so that it is in close agreement with the observed results. The type and value of the parameter will yield insight into the nature of the complexity that the parameter encapsulates.

The experimental system is envisaged to move from silicone into glass beads into sterile soil and finally into natural soil. The will allow stepwise consideration of the complexities associated with topology, chemical distribution and microbial distribution of the given environment respectively. In all experimental beaded soil systems the physical structure can be determined using the CT scanner and incorporated into the theoretical model as the underlying physical environment. The three-dimensional profiles of the mycelia in the transparent media such as silicone or glass beads can be accurately obtained using image analysis. However three-dimensional mycelial profiles in natural and sterile soil are anticipated to be obtained using low energy CT scanning although there is only tentative evidence that this can be done. Proof of concept work in this area will be required. Correction factors will be applied to match simulated and experimental outcomes when the experimental system moves from silicone, glass beads, sterile soil and finally natural soil.

The RISO system can also be used to address multi species interactions in the context of their response to a porous environment, and this is a step towards developing an ecology of fungi in soil. Fungal individuals can be distinguished using GFPs or dyes so that accurate biomass profiles of each fungal individual can be identified. We will again use the methodology of combining the glass-soil calibration for fungal interactions in a porous environment to derive an initial parameterization for the model of colony interactions. We can proceed by adding elements of complexity as before and determining the effect on the observed and simulated outcomes. Again, differences in these can be overcome by a correction factor, which will yield insight into the system under study.

7.5 Summary

This work arose out of a need to identify the underlying processes responsible for the observed phenotypes of individual and interacting vegetative mycelia. The originality of the approach is twofold:

1. The identification of the minimum rule set based on the physiological processes of fungal growth and development that results in emergence of realistic colony-scale structures. For the first time a biomass recycling mechanism was incorporated into a modelling framework for the growth and development of fungal mycelia;
2. the extension of the colony model to include interactions among mycelia, in which each fungal individual has an explicit representation in terms of trait values and spatial extent.

The resulting model has the following main features:

- Physiologically based, representing the processes of uptake, internal transport, recycling and growth of individual mycelia and interactions among mycelia.
- Potential parameterisation and validation of the trait values associated with the physiological processes from experimental data

The developed model has led to an understanding of the processes responsible for the growth of and interaction among fungal colonies and an understanding of those processes that are important for a given context. The model admits a number of assumptions for simplicity and the most obvious one is the lack of reproduction of the mycelia. We acknowledge incorporation of reproduction would reflect the lifecycle of the mycelium more completely as the dynamics of most fungi include some element of reproduction. However, the modelling undertaken was concerned with the vegetative growth of fungal colonies only. Clearly there are many more processes and details of real fungi that have been ignored in the modelling framework. Notwithstanding this, the results show that apparently complex behaviour can result from simple local rules spatially mediated by internal and external transport, and additional sophistication will not affect this general conclusion.

In the first instance, growth of individual and interacting mycelia was studied in simulated simple agar systems. The profile obtained encapsulates properties relating to growth rate, recycling, internal transport and combativeness. Agar systems are convenient experimental systems but are difficult to relate to real fungal systems as they admit key simplicities e.g. a non-structured, homogeneous resource base, which are not features of any natural environment in which fungi reside. Subsequently complexity was added to the underlying environment by incorporating a three-dimensions porous structure that mimics the physical architecture of a natural soil environment. Although this simulated set up is a step towards simulating a more realistic environment it is still very far removed from the real soil environment in which chemical and bacterial microbial heterogeneity is present.

One of the potential benefits of the model is its capacity to be used in a predictive sense. Since the model can incorporate explicitly measured experimental traits, i.e. it can include features like the production of general and antagonistic compounds, the model can be used to predict the effect of adding a certain fungal species to overall community structure in the target environment (usually soil) then it has potential to be utilised in bio control and remediation. If the model is to be used in this sense the underlying simulated environment needs to reflect more accurately the 'real' soil environment. This poses issues from the experimental side, as it is difficult to profile the behaviour of the mycelium in terms of response to physical architecture, microclimate, biological and chemical heterogeneities due to the opacity of the medium. By combining the theoretical work with an appropriate experimental system as discussed in Section 7.4, the theoretical model can be parameterised and validated, and may be used to determine the behaviour and ecology of soil fungi.

References

Allen, M., 1993. *The Ecology of Mycorrhizae*. Cambridge: Cambridge University Press.

Amir, R., Levanon, D., Hadar, Y & Chet, I., 1995. Factors affecting translocation and sclerotial formation in *Morchella esculenta*. *Experimental Mycology*, 19, 61-70.

Atkinson, K., 1978. *An Introduction to Numerical Analysis*. Wiley.

Batchelder, H.P. and Williams, R., 1989. Life History and population dynamics of *Metridia pacifica*: results from simulation modelling. *Ecological Modelling*, 48, 119-136.

Ben-Jacob, E., Shapira, Y., Becka, I., Raichman, N., Volman, V., Hulata., E and Baruchi, I., 2003. Communication-based regulated freedom of response in bacterial colonies. Physica *A* 330, 218 – 231.

Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirók A and Vicsek T., 1994. Generic modelling of cooperative growth patterns in bacterial colonies. *Nature,* 368 46-9

Berry, O. and Gleeson, D., 2005. Distinguishing historical fragmentation from a recent population decline – shrinking or pre-shrunk skink from New Zealand. *Biological Conservation,* 123, 197-210.

Bever, J.D., Schultz, P.A., Pringle, A. and Morton, J.B., 2001. Arbuscular Mycorrhizal Fungi: More diverse than meets the eye, and the ecological tale of why. *BioScience*, 51, 923 –931.

Bezzi, M. and Ciliberto, A., 2003. Modelling Growth of Filamentous Microorganisms. *Comments on Theoretical Biology*, 8, 563-585.

Blackwell, M., Vilgalys, R. and Taylor, J. W., 2005. Fungi. Eumycota: mushrooms, sac fungi, yeast, molds, rusts, smuts, etc. Version 14 February 2005 (under construction). Accessed 15/5/2006. Website: http://tolweb.org/Fungi/2377/2005.02.14 in The Tree of Life Web Project.

Boddy, L., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiology Ecology,* 31,185-194.

Boddy, L., 1999. Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. *Mycologia*, 91, 13-32.

Boddy, L. and Abdalla, S.H.M., 1998. Development of *Phanerochaete velutina* mycelial cord systems:effect of encounter of multiple colonised wood resources. *FEMS Microbiology Ecology*, 25, 257-269.

Boddy, L., 1993. Saprophytic cord-forming fungi: warfare strategies and other ecological aspects. *Mycological Research,* 97, 641-655.

Boddy, L., 1984. The micro-environment of basidiomycete mycelia in temperate deciduous woodlands. In: *Ecology and Physiology of the Fungal Mycelium* (ed. D. H. Jennings & A. D. M. Rayner). pp. 261-289, Cambridge: Cambridge University Press.

Boswell, G. P., Jacobs, H., Davidson, F., Gadd, G. and Ritz, K., 2003. Growth and Function of Fungal Mycelia in Heterogeneous Environments. *Bulletin of Mathematical Biology*, 65, 447-477.

Boswell, G. P., Jacobs, H., Davidson, F., Gadd, G. and Ritz, K., 2002. Functional consequences of nutrient translocation in mycelial fungi. *Journal of Theoretical Biology*, 217, 459-477.

Bown, J.L., 2000. *Issues of scale in individual-based models: Applications in fungal and plant community dynamics.* PhD thesis. University of Abertay Dundee.

Bown, J. L., Sturrock, C.J., Samson, W.B., Staines, H.J., Palfreyman, J.W., White, N.A., Ritz, K. and Crawford, J.W., 1999. Evidence for emergent behaviour in the community-scale dynamics of a fungal microcosm. *Proc. R. Soc. Lond. B*, 266,1947-1952.

Brasier, C.M., 1999. Fitness, Continuous Variation and Selection in Fungal Populations: an Ecological Perspective. In *Structure and Dynamics of Fungal Populations*. (Ed. L. Worral) pp 307-340. Kluwer Academic Publishers.

Brasier, C.M., 1996. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Ann. Sci. For*, 53, 347-358.

Brown, D. and Rothery, P.D., 1993. *Models in Biology: Mathematics, Statistics and Computing.* London:John Wiley.

Budgen, D., 2003. *Software Design*. Addison-Wesely Publishers. England.

Bull, A. T. and Trinci, A. J. P., 1977. The physiology and metabolic control of fungal growth. *Adv. Microb. Physiol,* 50, 1-84.

Cairney J.W.G and Burke, R.M., 1996. Physiological heterogeneity within fungal mycelia: an important concept for a functional understanding of ectomycorrhizal simbiosis. *New Phytologist*, 134, 685-95.

Cairney J. W. G., 1992. Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. *Mycological Research*, 96, 135-41.

Carlile M.J., Watkinson, S. C. and Gooday, G.W., 2001. *The fungi*, 2nd edn. San Diego: Academic Press.

Carlile, M. J., 1995. The success of the hypha and mycelium. In *The Growing Fungus* (ed. N. A. R. Gow and G. Gadd) pp. 1-17. London: Chapman & Hall .

Carlile, M. J. W., S.H and Gooday, G.W., 2001. The Fungi. 2nd ed.: Academic Press.

Cooke, R.C. and Rayner, A.D.M., 1984. *Ecology of Saprotrophic Fungi.* Longman, London and New York.

Crank, J. 1975. *The mathematics of diffusion.* 2nd Edition. Clarendon Press. Oxford.

Crutchfield, J.P. and Feldman, D.P., 2001. Regularities Unseen, Randomness Observed: Levels of Entropy Convergence, CHAOS. Santa Fe Institute Working Paper 01-02-012 and arXiv.org/abs/cond-mat/0102181

Crutchfield, J.P., 1994. The Calculi of Emergence: Computation, Dynamics, and Induction. *Physica D, 75, 11-54.* Santa Fe Institute Working Paper 94-03-016.

Davidson, F. A. and Olsson, S., 2000. Translocation Induced Outgrowth of Fungi in Nutrient-free Environments. *Journal of Theoretical Biology*, 205, 73-84.

Davidson, F. A., Sleeman, B.D., Rayner, A.D.M., Crawford, J.W. and Ritz, K., 1996. Context-dependent macroscopic patterns in growing and interacting mycelial networks. *Proceedings Royal Society B. Lond*, 263, 873-880.

Deacon, J., 2005. *Fungal Biology.* Blackwell Publishing. 4th Edition. London.

DeAngelis, DL, Phipps, MJ and Gross LJ., 1992. *Individual-Based Models and Approaches in Ecology: Populations, Communities and Ecosystems.* Chapman and Hall, New York.

Deutsch, A., Dress, A. and Rensing, L., 1993. Formation of morphological differentiation patterns in the ascomycete Neurospora crassa. *Mech. Dev.* 44, 17-31.

Dighton, J., 2003. *Fungi in Ecosystem Processes*. Volume 17. Marcel Dekker, Inc. New York.

Doohan, F., 2005. Fungal Pathogens of plants. In *Fungi: Biology and Applications*. (ed. K. Kavanagh), pp 219-250. London:John Wiley and Sons.

Dowson, C. G., Rayner, A. D. M. and Boddy, L., 1989. Spatial dynamics and interactions of the woodland fairy ring fungus, *Clitocybe nebularis*. *New Phytologist*, 111, 699-705.

Duchesne, L., 1994. Role of ectomycorrhizal fungi in biocontrol. In *Mycorrhizae and plant health*. (eds. F.L Pfleger and R.G Linderman), pp Minnesota: APS Press.

Dytham, C. and Shorrocks, B., 1992. Selection, patches and genetic variation: a CA modelling drosophila populations. *Evolutionary Ecology*, 6, 342-351.

Edelstein, L., 1982. The propagation of fungal colonies: A model for tissue growth. *Journal of Theoretical Biology,* 98, 679-701.

Edelstein, L. and Segel, L. A., 1983. Growth and metabolism in Mycelial Fungi. *Journal of Theoretical Biology*, 104,187-210.

Ellis, C.J., Crittenden, P.D., Scrimgeour, C.M. and Ashcroft, C.J., 2005. Translocation of 15N indicated nitrogen recycling in the mat-forming lichen *Cladonia portentosa*. *New Phytologist* 168, 423-434.

Ermentrout, G.B. and Edelstein-Keshet, L., 1993. Cellular automata approaches to biological modeling. *Journal of Theoretical Biology*, 160, 97-133.

Falconer, R.E., Bown, J.L., White, N.A. and Crawford, J.C., 2005. Biomass Recycling and the origin of phenotype in fungal mycelia. *Proc. Roy. Soc B. Lond*, 272,1727-1734.

Feeney, D., Crawford, J.W., Daniell, T.J., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M. & Young, I.M., 2006. 3D micro-organisation of the soil-root-microbe system. *FEMS Microbial Ecology* (accepted).

Fomina, M., Burford, EP and Gadd, G.M., 2005. Toxic metals and fungal communities. In *The Fungal Community Its Organization and role in the ecosystem.* (eds J. Dighton, J. White and P. Oudemans), pp 733-758 Boca Raton:Taylor & Francis Group.

Gadd, G., 2004. Mycotransformation of organic and inorganic substrates. *Mycologist*, 18: 60-70

Garret, D., 1981. *Soil fungi and soil fertility. An introduction to soil mycology. 2nd Edition.* Pergamon Press Oxford.

Goldwasser, L., Cook, J., and Silverman, E.D., 1994. The effects of variability on metapopulation dynamics and rates of invasion. *Ecology*, 75, 40-47.

Griffith, G.S. and Bardgett, R.D., 2000. Influence of resource unit distribution and quality on the activity of soil fungi in particulate medium. *New Phytologist, 148, 143-151.*

Griffith, GS and Rayner ADM., 1994. Interspecific interactions, mycelial morphogenesis and extracellular metabolite production in *Phebia radiate* (Aphyllophorales). *Nova Hedwigia*, 59, 331-334.

Griffin, D.H., 1994. *Fungal Physiology.* 2nd Edition. Wiley-Liss. New York.

Grimm, V., and Railsback, I., 2005. *Individual based modelling and ecology.* Princeton University Press.

Grindrod, P., 1991. *Patterns and Waves. The theory and applications of reaction-diffusion equations.* Oxford University Press. New York.

Halley, J. M., Robinson, C.H., Comins, H.N. and Dighton, J., 1996. Predicting straw decomposition by a four-species fungal community: a cellular automaton model. *Journal of Applied Ecology*, 33, 493-507.

Harris, K., Young, I.M., Gilligan, C.A., Otten, W. & Ritz, K., 2003. Effect of bulk density on the spatial organisation of the fungus *Rhizoctonia solani* in soil. *FEMS Microbiology Ecology*, 44, 45-56.

Hawksworth, D. L., 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.* 95, 641-655.

Hawksworth, D.L. and Rossman, AY., 1997 Where are all the undescribed fungi? *Phytopathology*, 87, 888-891.

Hiebler D., 1997. Stochastic spatial models: From Simulations to Mean Field and Local Structure Approximations. *Journal Theoretical Biology*, 187, 307-319.

Inghe, O., 1989. Genet and ramet survivorship under different mortality regimes - a cellular automata model. *Journal of Theoretical Biology*, 138, 257-270.

Jennings, D.H. and Lysek, G., 1996. *Fungal Biology.* Oxford: BIOS.

Kazakov, D and Sweet, M., 2000. Evolving the Game of Life In Lecture Notes in Computer Science In *Adaptive Agents and Multi-Agent Systems III: Adaptation and Multi-Agent Learning, (e*d.D. Kudenko, D. Kazakov and E, Alonso. Heidelberg: Springer.

Kennedy, D.M.& Duncan, J.M., 1995. A papillate *Phytophthora* species with specificity to *Rubus*. *Mycol Res*, 99 (1), 57-68.

Kneitel, JM and Chase, JM., 2004. Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecology Letters,* 7, 69-80.

Kreft, J.U., Picioreanu, C., Wimpenny, J.W.T., Van Loosdrecht, M.C.M., 2001. Individual-based modelling of biofilms. *Microbiology,* 147, 2897-2912.

Kreft, J.U., Booth, G., and Wimpenny, J.W.T., 1998. BacSim: a simulator for individual-based modelling of bacterial colony growth. *Microbiology,* 144, 3275-3287.

Levin, S., 1992. The problem of pattern and scale in ecology. *Ecology*, 73(6), 1943-1967.

Lesser, M.P., 2004. Experimental biology of coral reef ecosystems. *Journal of Experimental marine Biology and Ecology*, 300: 217-252.

Lewis, J.A., Larkin, R.P. and Rogers, D.L., 1998. A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soilless mix. *Plant Disease,* 85, 501–506.

Lindahl, S. and Olsson, S., 2004. Fungal Translocation – creating and responding to environmental heterogeneity. *Mycologist,* 18: 79-88.

Lomnicki A., 1999. Individual-based models and the individual-based approach to population ecology. *Ecological Modelling*,115, 191-198.

Lopez, J.M. and Jensen, H.J., 2002. A generic model of morphological changes in growing fungal colonies. *Phys. Rev. E*, 65, 021903.

Lysek, G., 1984. Physiology and ecology of rhythmic growth and sporulation in fungi. In *The Ecology and Physiology of the Fungal Mycelium,* (ed. D.H. Jennings and A.D.M. Rayner), pp383-417. Cambridge University Press.

Mason, R., 1997. Biomaterials – The Science and Biology Behind Biomaterials Research. *Materials World*, 5: 16-17.

Meskauskas, A., Fricker, D. and Moore, D., 2004. Simulating colonial growth of fungi with the Neighbour-Sensing model of hyphal growth. *Mycol. Res.,* 108, 1-16

Money, NP., 2004. The fungal dining habitat: a biomechanical perspective. *Mycologist,* 18, 71-76.

Murphy, R. and Horgan, K.A., 2005. Antibiotics, Enzymes and Chemical Commodities from Fungi. In *Fungi: Biology and Applications*. (ed. K. Kavanagh), pp 113-143. John Wiley and Sons.

Murray, J.D., 1989. *Mathematical Biology*. Springer Verlag. Heidelberg.

Newsham KK, Fitter AH, Watkinson AR., 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J Ecol* , 83, 991–1000

Nicolis, G., 1992. Physics of far-from-equilibrium systems and self organisation. In *The New Physics*. Ed. P. Davies. Cambridge University Press.

Okubo, A., 1980. *Diffusion and Ecological Problems: Mathematical Models*, Vol. 10 of Biomathematics, Springer-Verlag.

Olsson , S., 1999. Nutrient Translocation and electrical signalling in mycelia.In *The Fungal Colony*, (eds. N.A.R. Gow, G.D. Robson and G.M. Gadd), pp 25 – 48.Cambridge: Cambridge University Press.

Olsson, S. and Gray, S.N. 1998. Patterns and dynamics of ^{32}P-phosphate and labelled 2-aminoisobutyric acid (^{14}CX-AIB) translocation in intact basidiomycete mycelia. *FEMS Microbiology Ecology*, 26, 109-115.

Ortega, J.M. and Poole, J.W., 1981. *An introduction to numerical methods for differential equations,* Pitman, Boston.

Otten, W., Bailey, D. and Gilligan, C., 2004. Empirical evidence of spatial thresholds to control invasion of fungal parasites and saprotrophs. *New Phytologist.*,163, 125.

Otten, W., Gilligan, C A, Watts, C W, Dexter, A R and Hall, D.,1999. Continuity of air-filled pores and invasion thresholds for soil-borne fungal plant pathogen, *Rhizoctonia solani. Soil Biology and Biochemistry*, 31, 1803-1810.

Pachepsky, E. Taylor, T., and Jones, S., 2002. Mutualism promotes diversity and stability in a simple artificial ecosystem. *Artificial Life,* 8, 5-24.

Pachepsky, E., Crawford, J. W., Bown, J. L. and Squire, G. R., 2001. Towards a general theory of biodiversity. *Nature,* 410, 923-926.

Press, W., 1992 *NUMERICAL recipes in C : the art of scientific computing.* 2nd ed. Cambridge University Press.

Pringle, A and Taylor, JW., 2002. Fitness in filamentous fungi. *Trends Microbiol*, 10, 474-481

Prosser, J. I. and Trinci, A. J. P., 1979. A Model for Hyphal Growth and Branching. *Journal of General Microbiology*,111:153-164.

Rayner, A. D. M., Watkins, Z. R. and J.R., B., 1999. Self-integration - an emerging concept from the fungal mycelium. In *The Fungal Colony, (*ed. N.A.R. Gow, G.D. Robson and G.M. Gadd), pp. 1-24. Cambridge: Cambridge Press.

Rayner, A.D.M., Griffith, G.S. and Ainsworth, A.M., 1995. Mycelial Interconnectedness. In *The Growing Fungus,* (eds. Gow, N. and Gadd, G) London: Chapman and Hall.

Rayner, A.D.M., Griffith, G.S. and Howard, G.W., 1994. Induction of metabolic and morphogenetic changed during mycelial interactions among species of higher fungi. *Biochemical Society Transactions,* 22, 389-394

Rayner, A. D. M., 1994. Pattern-generating processes in fungal communities. In *Beyond the Biomass, (eds. K.* Ritz, J. Dighton and K.E, G.), pp 247-257. Wiley-Sayce.

Rayner, A. D.M., 1988. Life in a collective: lessons from the fungi. *New Sci.* 120, 49-53.

Rayner, A.D.M. and Webber, J., 1984. Interspecific mycelial interactions – an overview. In *The Ecology and Physiology of the Fungal Mycelium,* (ed. D.H. Jennings and A.D.M. Rayner), pp383-417. Cambridge University Press.

Read, N.D. and Hickey, P. C., 2001. The vesicle trafficking network and tip growth in fungal hyphae. In *Cell Biology of Plant and Fungal Tip Growth* (ed. A. Geitmann, M. Cresti and B. I. Heath) , pp. 137-148. Amsterdam: IOS Press.

Reaves J.L and Crawford R.H , 1994. In vitro colony interactions among species of *Trichoderma* with inference toward biological controls. *Pacific Northwest Research Station.* RP-474 94-040

Reynolds, C., 1995. Boids accessed 02/05/2004 13:55 http://www.red3d.com/cwr/boids/

Richard, S. A., Wilson, W. G. and Socolar, J.E.S., 1999. Selection for intermediate mortality and reproduction rates in a spatially structured population. *Roy. Soc. Proc. Lond. B*, 266, 2383-2388.

Richards, P., Mooij, W., and DeAngelis, D., 2004. Evaluating the effect of salinity on a simulated American crocodile population with applications to conservation and Everglades restoration. *Ecological Modelling*,180, 371– 394.

Ritz, K. and Young, I.M., 2004. Interactions between soil structure and fungi. *Mycologist*,18, 52-59.

Ritz. K., 2004. Fungal roles in transport processes in soils. Society for general Microbiology *The Roles and Impact of Fungi on Biogeochemical Cycles*. Eds. Gadd *et al.* Cambridge University Press.

Ritz. K., 1995. Growth Responses of some soil fungi to spatially heterogeneous nutrients. *FEMS Microbiology Ecology*, 16, 269-80.

Ruel, K. and Joseleau, J., 1991. Involvement of an Ectracellular Glucan Sheath during Degradation of *Populus* Wood by *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology,* 57, 374-384.

Shalizi ,C. R. , Shalizi, K. L. and Haslinger, R., 2004. Quantifying Self-Organization with Optimal Predictors. *Physical Review Letters*. 93, 118701.

Sharland, P. R. and Rayner, A. D. M., 1989a. Mycelial interactions in outcrossing populations of Hypoxylon. *Mycol, Res.* 93, 187-198.

Sharland, P. R. and Rayner, A. D. M., 1989b. Mycelial ontogeny and interactions in non-onterossing populations of Hypoxylon. *Mycol Res*, 93, 273-281.

Shea, K and Chesson, P., 2002. Community Ecology theory as a framework for biological invasions. *TRENDS in Ecology and Evolution*, 17, 170-176.

Sherratt, J.A. and Murray, J.D., 1990. Models of epidermal wound healing. *Proc. R. Soc. Lond. B*, 241, 29-36.

Silvertown, J., S. Holtier, J. Johnson, and P. Dale., 1992. Cellular automaton models of interspecific competition for space- the effect of pattern on process. *Journal of Ecology,* 80, 527-534.

Smith, M., Bruhn, J., and Anderson, J., 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature,* 356, 428-431

Stacey, A. J., Truscott, J. E. and Gilligan, C. A., 2001. Soil-borne fungal pathogens: scaling-up from hyphal to colony behaviour and the probability of disease transmission. *New Phytologist*, 150, 169-177.

Stahl, P.D. and Christensen, M., 1992. In vitro interactions among members of a soil microfungal community. *Soil Biology and Biochemistry,* 24: 309-316

Stamets, P. 2005. Mycelium Running: How mushrooms can help save the world. Ten Speed Press. California.

Stauffer, D. and Pandey, R., 1992. Simulation of demixing transition for binary fluid within a gel. *Journal Applied Physics Gen,* 1079-1085.

Stewart, I., 1997.*Does God Play Dice?: The new mathematics of Chaos.* Penguin Books Ltd. London.

Sturrock, C., Ritz, K., Samson, W., Bown, J.L., Staines, H., Palfreyman, J.W., Crawford, J.W. and White, N.A., 2002. The effect of fungal inoculum arrangement (scale and context) on emergent community dynamic development in an agar model system. *FEMS Micobiol. Ecol.*, 39, 9-16.

Swift, M., 2005. Human Impacts on Biodiversity and ecosystem services: An overview. In *The Fungal Community Its Organization and role in the ecosystem, (*Eds. J. Dighton, J. White and P. Oudemans). Boca Raton: P. Taylor & Francis Group.

Takenaka, A., 2001. Individual based model of a forest with spatial structure and gene flow. In *Present and future of modelling global environment change: Toward Integrated Modeling.* (Ed.T. Matsuna and H. Kida, H) pp 415 - 420.

Tilman, D., 1994. Competition and biodiversity in spatially structured habitats. *Ecology*, 75(1) 2-16

Trefil, J., 2002. *Cassell's Laws of Nature.* Orion Press. New York.

Vogt, K.A., Grier, C.C., Edmonds, R.L. and Meier, C.E., 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis.* Forbes ecosystems in western Washington. *Ecology*, 63:370-380.

Wakelin, S.A., Sivasithamparam, K., Cole, A.L.J. and Skipp, R.A., 1999. Saprophytic growth in soil of a strain of *Trichoderma koningii. New Zealand Journal of Agricultural Research,* 42, 337-345.

Walker G and White N.A., 2005. Introduction to fungal physiology. In *Fungi: Biology and Applications*, (ed K. Kavanagh) pp 1-34. John Wiley and Sons.

Watanabe, T., 2002. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species.* 2nd Edition CRC Press. London.

Watkinson, S.C., Boddy, L., Burton, K.S., Darrah, P.R.,Eastwood, D., Fricker, M.D. and Tlalka, M., 2005. New approaches to investigating the function of mycelial networks. *Mycologist*, 19, 11-17.

Whipps, J. M. & Davies, K. G., 2000. Success in biocontrol of plant pathogens and nematodes by microorganisms. In: *Biological Control: Measures of Success*, eds G. Gurr & S.D. Wratten, Dordrecht, The Netherlands: Kluwer, pp 231-269.

Whipps, J.M., 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*, 82, 1198-1227.

White, N. A., 2003. The importance of wood-decay fungi in forest ecosystems. In: editor. *Fungal biotechnology in Agriculture, Food and Environmental Applications*, (ed. D.K. Arora.) pp. 375-392.New York: Marcel Dekker.

White, N. A., Sturrock, C., Ritz, K., Samson, W.B., Bown, J., Staines, H.J., Palfreyman, J.W. and Crawford, J., 1998, Interspecific fungal interactions in spatially heterogeneous systems. *FEMS Microbial Ecology*, 27, 21-32.

White, N. and Boddy, L., 1992. Extracelluar enzyme location during interspecific fungal interactions. *FEMS Microbial Ecology*, 98, 75-80

White, S. and White, J., 2005. Applications of biological control in resistant host–pathogen systems. *Mathematical Medicine and Biology*, 22, 3, 227-245.

Wolf, T. and Holvoet, T., 2004. Emergence Versus Self-Organisation: Different concepts but Promising when combined. In *Engineering Self-Organising Systems: Methodologies and Applications*. pp 1-15 Springer Berlin. Eds. Brueckner. S, Giovanna, D., Karageorgos, A and R Hagpal.

Wolfe,D., 2001.*Tales from the underground. A natural history of Subterranean Life.* Perseus Publishing. Cambridge, Massachusetts.

Wolfram, S., 2002. *A New Kind of Science*. Wolfram Media. New York

Wolfram, S., 1986. *Theory and applications of cellular automata*. World Scientific, Singapore.

Young, I. and Crawford, J., 2004. Interactions and Self-Organization in the Soil-Microbe Complex. *Science*, 304,1634-1637.

Young, I.M. and Ritz, K., 2005. The habitat of soil microbes. In *Biological Diversity and Function in Soils*, ed. R. D. Bardgett, D. W. Hopkins and M. B. Usher, pp. in press. Cambridge University.

Appendix A. Model Algorithms

Fungal phenotype model

Initialise environment

Initialise trait values

Begin main

For cycles = 1 → maxcycles

For the fungal individual (for each cell containing biomass)

Uptake resource*

Update mobile field*

Update immobile non insulated biomass*

Update immobile insulated biomass*

End main

Begin uptake resource

Determine uptake*

End uptake resource

Begin determine uptake

If total requested uptake > external resource

Uptake = total requested uptake

If total requested uptake < external resource

Uptake = external resource

End determine uptake

Begin update mobile field

Determine mobile biomass derived from uptake*

Derive immobilization component for immobile insulated and non-insulated biomass*

Derive mobilization component for immobile insulated and non-insulated biomass *

Determine Diffusion coefficient for mobile field*

Diffuse mobile biomass component

End update mobile field

Begin mobile biomass derived from uptake

Mobile biomass = uptake

End mobile biomass derived from uptake

Begin derive insulated and non-insulated immobilization components

Insulated immobilization=Insulated immobilisation trait*(mobile/immobile biomass)$^{\text{nonlinear trait}}$

Non-insulated immobilization=Non-insulated immobilisation trait*(mobile/immobile biomass)$^{\text{nonlinear trait}}$

End derive insulated and non-insulated immobilization components

Begin derive insulated and non-insulated mobilization components

Insulated mobilization=Insulated mobilisation trait*(mobile/immobile biomass

Non-insulated mobilization=Non-insulated mobilisation trait*(mobile/immobile biomass)^{t}

End derive insulated and non-insulated mobilisation components

Begin determine diffusion coefficient mobile field

If mobile biomass field > 0.01*external_resource

$D_n=0$

Else

$D_n=D_b$ where D_b = diffusion coefficient for biomass growth

End determine diffusion coefficient mobile field

Begin update non-insulated biomass

Add non-insulated immobilisation component

Subtract non-insulated mobilisation component

Subtract percentage converted into insulated biomass*

Determine diffusion coefficient*

Diffuse non-insulated biomass

End update non-insulated biomass

Begin subtract percentage converted into insulated biomass (%)

*% = conversion_trait*non- insulated biomass*

Begin subtract percentage converted into insulated biomass

Begin determine diffusion coefficient non insulated biomass field

If pore

$D_b = D_b$

Else

$D_b = 0$

End determine diffusion coefficient non insulated biomass field

Begin update insulated biomass

Add immobilisation component

Add percentage obtained from non-insulated biomass

Subtract mobilisation component

End update insulated biomass

Fungal Interaction Phenotype Model

Initialise environment

Initialise trait values

Begin main

For cycles = 1 → maxcycles

For each fungal individual and for each cell containing biomass

Uptake resource*

Update mobile field*

Update global enzyme field*

Update immobile non-insulated biomass*

Update immobile insulated biomass*

End main

Update resource and update insulated biomass are as detailed in single phenotype model

Begin Update mobile field

Determine mobile biomass derived from uptake*

Determine extra field derived from mobile biomass*

Derive immobilization component for immobile insulated and non-insulated biomass*

Derive mobilization component for immobile insulated and non-insulated biomass *

Determine Diffusion coefficient for mobile field*

Diffuse mobile biomass component

End Update mobile field

All sub processes in update mobile filed are as detailed in Single phenotype model apart from determine extra field derived from mobile biomass.

Begin extra field derived from mobile biomass

Enzyme field =enzyme field + efficency*enzyme_production_trait*mobile_field

End extra field derived from mobile biomass

Begin Update Global Enzyme field

Global_enzyme= Global_enzyme + individual extra enzyme

End Update Global Enzyme field

Begin update non-insulated biomass

Add non-insulated immobilisation component

Subtract non-insulated mobilisation component

Subtract percentage converted into insulated biomass*

Determine stuff to be lysised*

Determine diffusion coefficient*

Diffuse non-insulated biomass

End update non-insulated biomass

Percentage converted into insulated biomass is as detailed in single phenotype model

Begin determine stuff to be lysised

If global enzyme > individual enzyme

Mob_ni = 0.9

Immob_ni = 0.0

Mob_in = 0.9

Immob_in = 0.0

If mobile biomass >> biomass tot

Environmental resource=Environmental resource + mobile biomass

End determine stuff to be lysised

Begin determine diffusion coefficient

If global extra field > individual extra field

D=0

Else

D=Db

End determine diffusion coefficient

Appendix B. Model Prototypes

B.1 Prototype I

B.1.1 Model Formulation

Invasion Percolation models have been used to describe the dynamics of fluids moving through porous solids. In such models, fluid enters some spatially structured network at a given point and progresses through that network, expanding from the perimeter, following the path of least resistance. This form of model can be used to represent the growth of fungal colonies in soil, where the values associated with the resistance represent a porosity measure relating to the soil pore network and the movement of fluid can be considered analogous to fungal growth.

The underlying porous medium (soil lattice) is obtained by generating spatially correlated lattices using the recursive Random Midpoint Displacement Algorithm. In one dimension this algorithm works by:

- Maintaining an interval with endpoints (x_0, y_0) and (x_1, y_1).
- Divide this interval in half.
- Randomly displace the midpoint to (x_{mid}, y_{mid}) where $x_{mid} = (x_0 + x_1)/2$ and and $y_{mid} = (y_0 + y_1)/2 + \Delta$ where Δ is chosen at random to be a value from the Gaussian distribution with mean 0 and variance σ .
- Recur on the left and right intervals, decreasing the variance by a factor of 2.

For a 2-Dimensional brownian fractional surface, the Δ value must be generated from a Gaussian distribution and in the i-th iteration the variation (σ_i) will be modified according to: $\sigma_i^2 = (1/2^{2H(i+1)})\ \sigma^2$ where H is the Hurst exponent. From this equation the first iteration has the biggest effect on the resulting shape of the surface and the influence of other iterations decrease. Fractional brownian motion (fbM) is a randomized fractal that generates realistic models for many naturally rugged shapes and surfaces. Soil is known to have fractal charecteristics and fractal models have been used to simulate the structural heterogeneities of soil (Young & Crawford 2004) in two and three dimensions. The fractal dimension (D) of an approximated fbM surface is derived from the Hurst

exponent and is defined as D = 3 – H. Different fractal dimensions can correspond to different soil bulk porosities.

The expansion of fungi through the simulated 2-D soil structure is determined by the presence or absence of hyphal biomass in a local neighbourhood. If a cell contains biomass then the cell in the local neighbourhood with the highest porosity measure will become occupied by the colony at the next iteration. Quantitative values for biomass densities are not associated with this prototype.

B.1.2 Results

Underlying structures

(a) (b)

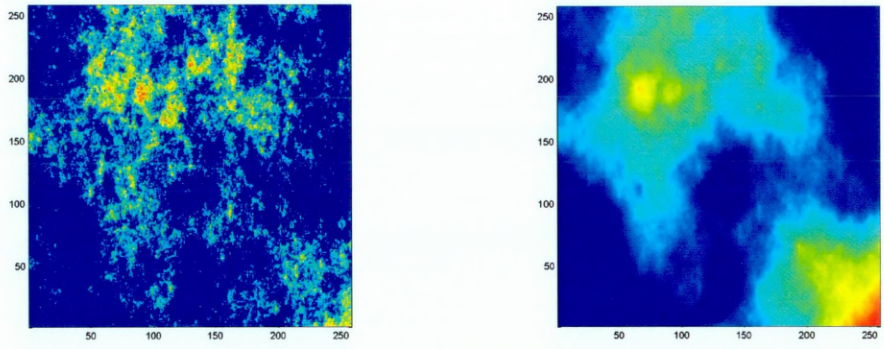


Figure B. 1 simulated structural distributions obtained using a) H = 0.1, D = 2.9; b) H = 0.9, D = 2.1

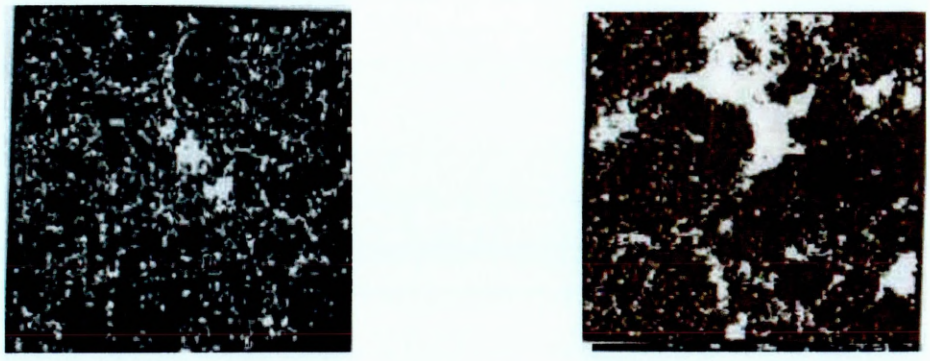


Figure B. 2 Experimentally derived 2D soil thin sections (Young and Crawford 2004)

The structures generated (Fig. B.1) are similar to 2-D thin sections of soil, which can now be obtained experimentally (Fig. B.2). At the time of developing Prototype I this experimental data was

not available. The dark blue colour of Fig. B.1 represents soil particles/phases where the colony cannot grow. The pale blue to red regions correspond to pores where the mycelium can grow. From the plots above it is evident that pores vary in spatial scale; in Fig. B.1 (b) there exists two relatively large pores and in Fig. B.1 (a) a greater number of smaller pores exist.

Biomass distributions

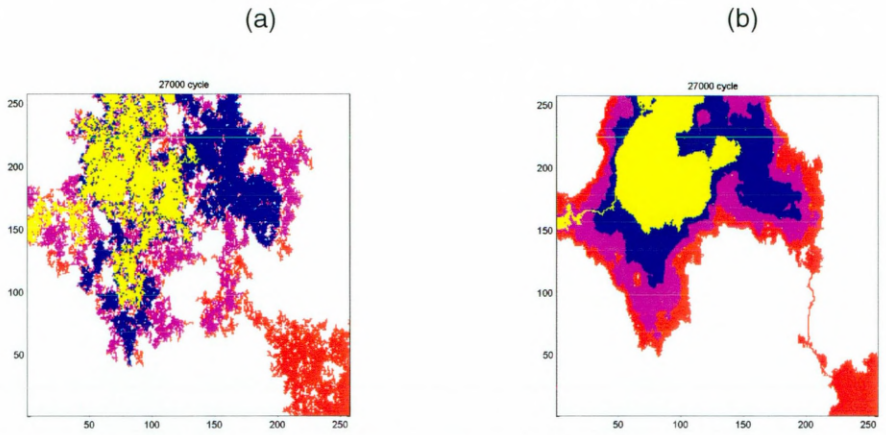


Figure B. 3 Simulated biomass distributions where fungi are grown through the simulated structures of B.1 (a) and (b) respectively

The corresponding biomass distributions using the simulated structures of Fig. B.1.The biomass enters from the middle left. The colours represent the length of time pores have been filled: those filled for the longest time yellow, then blue, pink and finally red. The figures illustrate a low and high density filling of the pore network respectively. The biomass distributions demonstrate that the colony growth is constrained by the physical architecture.

B.1.3 Discussion

The phenomenological model produces biomass distributions relating to fungal colony dynamics in soil, but it does not provide an understanding of the mechanisms that are responsible for the observed phenomena. To obtain an appreciation of these mechanisms a process-based model is required. Process-based models aim to describe the system being modelled by a set of physical laws. In terms of fungal colony dynamics, colonies can be described by physiological traits that govern physiological processes. These physiological processes are described by a set of mathematical equations, which are believed to drive the dynamics of the system under study. The

traits are characterized by experimentally derived parameters and so an investigation into which parameters are responsible for a particular dynamic can be conducted. A process-based model allows a number of scenarios to be investigated with relative ease and the formulation of a hypothesis. This hypothesis can then be validated by the experimental system.

B. 2 Prototype II

B.2.1 Model Formulation

This prototype is process-based and the following physiological processes are thought to govern the dynamics of the colony:

- Uptake of nutrients
- Redistribution of nutrients
- Growth
- Uptake of nutrients

B.2.1.1 Uptake

The uptake process is described by:

$$u = u_t * b * n_e$$

where u_t is the uptake trait

b is the amount of biomass

n_e is the amount of external resource

The uptake trait reflects the suitability of nutrients as well as primary resource capture. The uptake trait increases the rate of uptake of nutrients from the environment, therefore reducing the amount available to competing organisms. Once the nutrients are taken up by the colony they are converted into mobile biomass, which can be used by the mycelium. The equation also reflects via a linear relationship that primary resource capture is maximised through increased biomass and external resource.

B.2.1.2 Redistribution of mobile biomass

common process in biology and is commonly used to model bacterial growth and animal Part of the redistribution process is governed by a passive diffusion equation. Diffusion is a movement. Diffusion describes the movement of molecules along a concentration gradient, i.e. from areas of

high concentration to low concentration until equilibrium is achieved. In this case the molecules (mobile biomass), which have been derived from uptake can be transported within the networked fungal colony. Some parts of the colony will have a greater concentration of mobile biomass in areas where uptake is high. The diffusive process reduces this concentration gradient. The diffusive process can be described mathematically by:

$$\frac{\partial n}{\partial t} = D\frac{\partial^2 n}{\partial x^2} + D\frac{\partial^2 n}{\partial y^2}$$

where n represents the concentration of mobile biomass

x and y are the variables representing 2-D space

D is a constant diffusion coefficient

The diffusion coefficient has units (length)^2 (time^{-1}) and is the constant of proportionality. The diffusion coefficient controls the rate of movement of mobile biomass. In order to approximate this equation numerically the finite-difference, explicit method is used. This involves discretising the spatial and temporal scales and applying the Taylor's series.

The redistribution process also comprises of a process that is required to redistribute the mobile biomass to regions that are undergoing exploitation (convection). This process reflects the translocation of nutrients in the direction of growth when a new resource is encountered. This increases the rate of exploitation further.

B.2.1.3 Growth

Growth of the hyphal biomass is simulated using a passive diffusion mechanism as in the redistribution process. The diffusion coefficient governing the colony extension rate can be assigned in accordance with the underlying growth environment. For a homogeneous non-structured environment the diffusion coefficient is constant, however, for a structured environment such as soil the diffusion coefficient can be a function of porosity.

B.2.2.3 Environment

The environment used in the following simulations was simulated as in Prototype 1. The concentration gradient corresponds to the porosity and thus the diffusion coefficient of the soil structure. The resource is distributed within the pore network so it can be accessible to the mycelium.

Determining the effect of individual traits on a resultant phenomenon is difficult when the traits are interacting. Relationship between the parameter settings and resultant phenomenon can be non-linear. If non-linear interactions are taking place the emergent behaviour of the whole is not simply the additions of the parts, the influences are not separable, and it is often difficult to determine a particular parameters effect. To determine the effect of the traits a Genetic Algorithm (GA) is used and will also determine the trait set for optimal growth in a soil environmnet. GAs are numerical optimisation algorithms inspired by both natural selection and natural genetics. They involve using a population of solutions to solve optimisation problems.

B.2.2 Genetic Algorithm Framework

A GA is used to determine which set of traits lead to an optimal individual in a soil-like environment. An optimal fungal colony during the resource colonisation stage of the lifecycle is defined by its ability to exploit the environment. The purpose of the GA is to identify which trait set leads to effective resource exploitation. The GA will also highlight which traits are driving the system.

The fungal individual is described by four traits that control the behaviour of the processes described in B.2.1. These traits and their associated ranges, in brackets, are:

- u_t - uptake trait (0 - 1)
- cl - convection length (0 - 5)
- cs - convection strength (0 - 1)
- Dr – diffusion coefficient for mobile biomass (0 - 4)

The GA initially generates a population with random values drawn from the ranges above for each of the traits. This trait set governs the physiological behaviour of the fungal individual. Each of these individuals is then grown through a simulated soil environment and the length of time it takes to utilise all resource is used as the fitness metric. The GA applies a cross over operator that

promotes mixing fragments of better solutions to form new, on average even better solutions which will then be used in the following generation. The GA also uses a mutation operator to maintain diversity within the solutions. The GA operators (crossover and mutation) are then applied every iteration until the maximum number of generations is attained. One disadvantage of fitness proportional selection is that it does not guarantee selection of the fittest individual to be carried into the next generation. Unless the fittest is much fitter it will occasionally not be selected therefore the best solution to a problem may occasionally be thrown away. This may be advantageous as it slows down the algorithm forcing it to search more space before convergence. The main difficulty in implementing a GA is getting the balance between exploitation and exploration correct. The search speed can be greatly increased by not losing the best or elite member between generations. This however may focus the search in a local area therefore not exploring the search space enough. Not only is the elite member selected but a copy of it doesn't become disrupted by crossover or mutation. The pseudo code for the GA is given below in Box B.1.

1.Generate an initial population of random binary strings of length $\sum_{k=1}^{4} l_k$, where 4 is the number of Unknowns and l_k the length of the binary string required by an unknown.

2.Decode each individual, i, within the population to integers $z_{i,k}$ and then to real numbers $r_{i,k}$, to obtain the unknown parameters.

3.Run each individual through the simulated environment and determine the fitness f_i, where a better solution implies a higher fitness.

4. Select, by using fitness proportional selection, pairs of individuals and apply with probability P_c single point crossover. Repeat until a new population is formed.

5. Apply the mutation operator to every individual in the temporary population by stepping bit wise through each string, occasionally flipping a 0 or a 1. The probability of any bit mutating is given by P_m and is typically very small.

6. If the temporary population does not contain a copy of an individual with at least the fitness of the elite (fittest) member, replace (at random) one member of the temporary population with the elite member.

7. Replace the old population by the new generation.

8. Increment by 1, the generation counter and repeat from step 2 until G generations have elapsed.

Box B. 1 pseudo code for Genetic Algorithm

The initial population of strings (length 16) will be randomly created representing the four unknowns with 4-bit encoding. Linear mapping is used to map the population of integers, (calculated from the binary representation of the string) to real numbers as in step 2 of box C 1. The table below shows the decoding and range of the four parameters. A fungal individual can be represented by the string

0011011101110001 which corresponds to parameter values of 0.8, 0.472, 0.47 and 1 for Dr, u_t, cs and cl respectively.

4 bit string	Dr	u_t	cs	cl	Integer value, (z)
0000	0	0.01	0	0	0
0001	0.27	0.076	0.067	1	1
0010	0.54	0.142	0.14	1	2
0011	0.8	0.208	0.2	1	3
0100	1.07	0.274	0.27	2	4
0101	1.34	0.34	0.33	2	5
0110	1.6	0.406	0.4	2	6
0111	1.87	0.472	0.47	3	7
1000	2.14	0.538	0.53	3	8
1001	2.4	0.604	0.6	3	9
1010	2.67	0.67	0.67	4	10
1011	2.93	0.736	0.73	4	11
1100	3.2	0.802	0.8	4	12
1101	3.47	0.868	0.87	5	13
1110	3.74	0.934	0.93	5	14
1111	4	1	1	5	15

Table B. 1decoding of bit representation to parameter values

B.2.3 Results

Below is the biomass distributions (Fig. B.4) and the distribution of diffusion coefficients based on the simulated 2D soil structure (Fig. B.5). The distribution of biomass will be dependent on the physiological traits of the individual. The Figures B.4. (a) and (b) differ in the trait values for the fungal colony. In Fig. B.4 (a) the uptake trait is higher resulting in a higher biomass density than in Fig. B.4. (b). In Fig. b the diffusion coefficient for the mobile biomass is high which increases the individual's space filling capacity. The length of time it takes for an individual to exploit all of the available resource is also dependent on the physiological traits for that individual.

(a) (b)

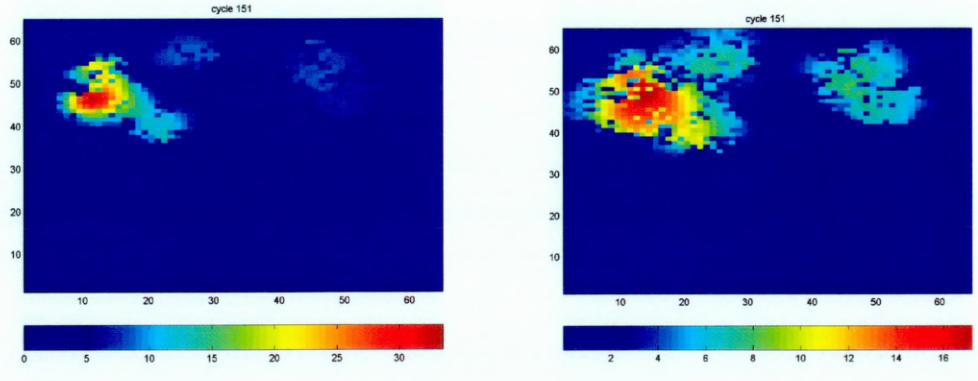


Figure B. 4 Biomass distributions of two different fungal individuals. The traits in (a) are [u_t = 1.0, cs = 0.0, Dr = 0.0, cl = 0.0] and in (b) are [u_t = 0.01, cs = 1.0, Dr = 4.0, cl = 0.0]

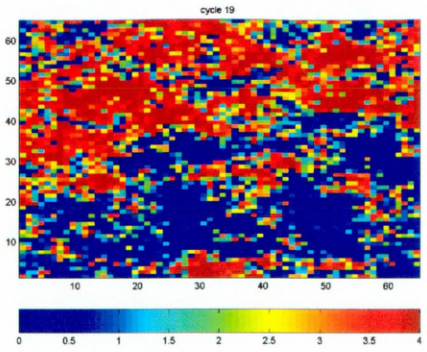


Figure B. 5 Distribution of diffusion coefficients that govern fungal hyphal growth

B.2.4 Discussion

The formulation and implementation of the convective redistribution of mobile biomass towards freshly exploiting regions of the colony was not satisfactory for two reasons. First, the convective process did not improve the fitness of the colony; it can be considered a redundant process. Second, the implementation of the convection process imposed some kind of knowledge regarding the overall state of the colony, which is not true of natural fungal systems. The fungal network is decentralized just like the World Wide Web. There is no region capable of exerting control over the rest of the network. The implementation of the convection process infers a central intelligence that is able to detect where the highest mobile biomass peak is located within the colony. It is towards this peak that all other mobile biomass is directed to varying degrees dependent on the convection strength trait. In natural systems fungi will respond to the environment, and the processes that control the growth and development of the colony, emerge as a consequence of this interaction with the environment. Growth patterns are forged by the environment as well as genotype with no centralized control.

This prototype although more realistic than the original still lacks a fundamental process which is crucial for fungi to survive in complex environments. A process that allows the mycelium to reutilise 'aged' biomass is thought vital for the survival of fungal colonies in heterogeneous complex environments. It is this recycling process that allows the fungus to change form by reallocating older parts of the colony. The model should also include a loss in terms of metabolic costs associated with processes, which will add realism to the model.

The final prototype presented in Chapter 3 was re-designed to include a new formulation of the redistribution process. This still reflects the translocation of nutrients in the direction of growth when a new resource is encountered. The next prototype should also include a recycling process and metabolic loses. All processes should be implemented in a manner which assumes no centralized control i.e. emergence of all colony responses are a consequence of interaction with the environment.

Appendix C. 1-Dimensional Finite Difference Method - Forward Time Central Space (FTCS)

The forward-time-central-space finite difference method is an explicit method as the concentrations of the cells at the next time step are obtained by explicit formula in terms of the known values at the previous time step. The first step in discretizing the continuous equation is to replace the differential operator ∂ with the discrete analog Δ. Consider the 1D case where a plane is discretised in terms of time and space and the horizontal and vertical axes correspond to space and time respectively.

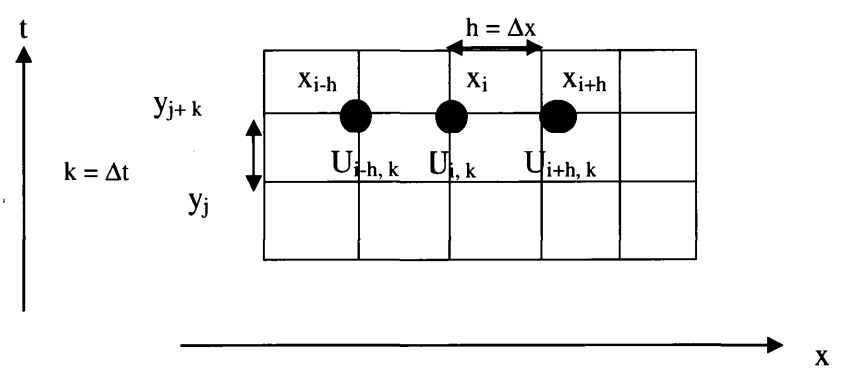


Figure C. 1 2-dimensional space discretized in terms of spatial (x) and temporal (y) domains.

Now, we define the central differences of du at point x_i:

$\Delta u_i = u_{i+h} - u_{i-h}$ central difference

The central difference approximation of the tangent of the slope (the derivative) at x_i is:

$$\frac{\partial u}{\partial x} = \frac{\Delta u}{\Delta x} = \frac{u_{i+h} - u_{i-h}}{x_{i+h} - x_{i-h}} \qquad \text{(a)}$$

The Taylor series relates the function u(x) to values at adjacent points (x+h or x-h) and the derivatives of u(x). By using Taylor's series in the direction of x but keeping t constant we can write

approximations for u(x+h) and u(x-h) i.e. the concentrations at the positions to the left and right of u(x).

$$u_{i+h,j} = u_{i,j} + h\left(\frac{\partial u}{\partial x}\right)_{i,j} + \frac{1}{2}(h)^2\left(\frac{\partial^2 u}{\partial x^2}\right)_{i,j} + ...R_n, \quad \text{(b)}$$

$$u_{i-h,j} = u_{i,j} - h\left(\frac{\partial u}{\partial x}\right)_{i,j} + \frac{1}{2}(h)^2\left(\frac{\partial^2 u}{\partial x^2}\right)_{i,j} + ...R_n, \quad \text{(c)}$$

R_n term is a remainder term to account for all terms from the second order to infinity, R_n corresponds to the truncation error. Adding and rearranging for the second spatial derivative gives us the 2nd order central difference approximation of the 2nd derivative:

$$\frac{\partial^2 u}{\partial x^2} = \frac{u_{i+h,j} - 2u_{i,j} + u_{i-h,j}}{h^2} \quad \text{(d)}$$

Defining the forward difference approximation at the time derivative gives:

$$\frac{\partial u}{\partial t} = \frac{u_{i,j+k} - u_{i,j}}{k} \quad \text{(e)}$$

Now using Taylor's series in the direction of t but keeping x constant we can obtain an approximation for f(x+k) i.e. the concentration at the next time step by:

$$u_{i,j+k} = u_{i,j} + k\left(\frac{\partial u}{\partial t}\right)_{i,j} + \frac{1}{2}(k)^2\left(\frac{\partial^2 u}{\partial t^2}\right)_{i,j} + ..., \quad \text{(f)}$$

Rearranging for $\frac{\partial u}{\partial t}$ and ignoring 2nd order terms provides:

$$\frac{\partial u}{\partial t} = \frac{u_{i,j+k} - u_{i,j}}{k} \quad \text{(g)}$$

Substituting the expressions of (d) and (g) into $\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2}$ gives us our finite difference approximation for the continuous diffusion equation.

$$\frac{u_{i,j+k} - u_{i,j}}{k} = D\frac{u_{x+h,j} - 2u_{x,j} + u_{x-h,j}}{h^2} \quad \text{(h)}$$

Upon rearranging for the concentration at the next time step ($U_{i,j+1}$) :

$$u_{i,j+k} = u_{i,j}\left(1 - \frac{2kD}{h^2}\right) + \frac{kD}{h^2}\left(u_{x+h,j} + u_{x-h,j}\right)... \quad \text{(i)}$$

In 2 dimensions this becomes:

$$\frac{u^{m+1}{}_{x,y} - u^{m}{}_{x,y}}{k} = \frac{D}{2*h^2}\left[u^{m}{}_{x+1,y} + u^{m}{}_{x-1,y} + u^{m}{}_{x,y+1} + u^{m}{}_{x,y-1} - 4*u^{m}{}_{x,y}\right] \quad \text{(j)}$$

and upon rearranging (j):

$$u_{x,y}{}^{m+1} = u_{x,y}{}^{m}\left[1 - \frac{4kD}{h^2}\right] + \frac{kD}{h^2}\left(u_{x+h,y} + u_{x-h,y} + u_{x,y+h} + u_{x,y-h}\right) \quad \text{(k)}$$

where x and y correspond to the spatial dimensions and the superscript, m, represents the temporal scale.

Notes on Accuracy

The Taylor series specifies the value of a function at one point, x, in terms of the value of the function and its derivatives at a reference point, a. By using the Taylor's series to get an expression for the first derivative of the forward difference with respect to time in terms of difference operators as in (f) we ignore all terms greater than the first order. The forward difference approximation is therefore 1st order accurate. For the central difference approximation with respect to space, expressions (b) and (c), we ignore all terms greater than second order. The central difference approximation is therefore 2nd order accurate.

Appendix D. Crank Nicholson Method

The Crank Nicholson method is the average of the explicit and the implicit method.

The explicit expression (note the values for conc. at the current time step are m):

$$\frac{u^{m+1}{}_{i,j}-u^{m}{}_{i,j}}{k}=\frac{D}{2*h^2}\left[u^{m}{}_{i+1,j}+u^{m}{}_{i-1,j}+u^{m}{}_{i,j+1}+u^{m}{}_{i,j-1}-4*u^{m}{}_{i,j}\right](a)$$

The implicit expression (note the values for conc. at the future time step are m+1):

$$\frac{u^{m+1}{}_{i,j}-u^{m}{}_{i,j}}{k}=\frac{D}{2*h^2}\left[u^{m+1}{}_{i+1,j}+u^{m+1}{}_{i-1,j}+u^{m+1}{}_{i,j+1}+u^{m+1}{}_{i,j-1}-4*u^{m+1}{}_{i,j}\right](b)$$

Taking the average of (a) and (b) gives:

$$u^{m+1}{}_{i,j}-u^{m}{}_{i,j}=\frac{D*k}{4*h^2}\left[u^{m}{}_{i+1,j}+u^{m}{}_{i-1,j}+u^{m}{}_{i,j+1}+u^{m}{}_{i,j-1}-4*u^{m}{}_{i,j}+u^{m+1}{}_{i+1,j}+u^{m+1}{}_{i-1,j}+u^{m+1}{}_{i,j+1}+u^{m+1}{}_{i,j-1}-4*u^{m+1}{}_{i,j}\right](c)$$

Substituting $\mu=\frac{D*k}{4*h^2}$ and rearranging gives:

$$u^{m+1}{}_{i,j}(1+4\mu)-\mu(u^{m+1}{}_{i+1,j}-u^{m+1}{}_{i-1,j}-u^{m+1}{}_{i,j+1}-u^{m+1}{}_{i,j-1})=u^{m}{}_{i,j}(1-4\mu)+\mu(u^{m}{}_{i+1,j}+u^{m}{}_{i-1,j}+u^{m}{}_{i,j+1}+u^{m}{}_{i,j-1})$$

Box D. 1Steps of the two dimensional CN method

The formula has been arranged in (d) with known terms on the RHS and unknown on the LHS. This results in a set of M*N simultaneous equations where M and N are the number of grid points in the x and y direction that need to be solved for the concentrations at the next time step. The systems of simultaneous equations are solved using an iterative method e.g. Successive Over Relaxation (SOR). The algorithm used to solve the nonlinear diffusion process via the CN and SOR iterative method is outlined below.

Crank Nicholson algorithm

1. Initialise A, B and μ coefficient matrix
2. Determine interior nodes of the coefficient matrix, these values determine the quantity to be diffused into and out of a cell.

a. $\mu = \frac{D * k}{4 * h^2}$

b. A = (1+4μ)

c. B = (1-4μ)

3. Determine the value C at time m i.e. the RHS of the equation below which is based on known values:

$$u^{m+1}{}_{i,j}(1+4\mu) - \mu(u^{m+1}{}_{i+1,j} - u^{m+1}{}_{i-1,j} - u^{m+1}{}_{i,j+1} - u^{m+1}{}_{i,j-1}) = u^{m}{}_{i,j}(1-4\mu) + \mu(u^{m}{}_{i+1,j} + u^{m}{}_{i-1,j} + u^{m}{}_{i,j+1} +$$

4. Use this known value to solve for the unknowns of the LHS of equation 3 above, using an iterative method.

$$u^{m+1}{}_{i,j}(1+4\mu) - \mu(u^{m+1}{}_{i+1,j} - u^{m+1}{}_{i-1,j} - u^{m+1}{}_{i,j+1} - u^{m+1}{}_{i,j-1}) = \text{C (a)}$$

$$u^{m+1}{}_{i,j}(1+4\mu) = C + \mu(u^{m+1}{}_{i+1,j} - u^{m+1}{}_{i-1,j} - u^{m+1}{}_{i,j+1} - u^{m+1}{}_{i,j-1}) \text{ (b)}$$

$$u^{m+1}{}_{i,j} = [C + \mu(u^{m+1}{}_{i+1,j} - u^{m+1}{}_{i-1,j} - u^{m+1}{}_{i,j+1} - u^{m+1}{}_{i,j-1})]/(1+4\mu)(c)$$

5. Use an iterative method to solve this set of simultaneous equations, such as Gauss-Seidel or SOR

SOR Algorithm

1. Make an initial guess u_{ij} for all interior points (i, j), this is normally zero
2. Define a scalar w (0 < w < 2),
3. Apply equation 4(c) of CN algorithm above to all interior points and call it u'_{ij}
4. Apply successive over-relaxation, $u'_{ij} = w^* u'_{ij} + (1-w)^* u_{ij}$
5. Stop if prescribed convergence threshold is reached otherwise continue on next step
6. Go to Step 2

Testing of the Crank Nicholson method

The Crank Nicholson method was tested against the already tested explicit method to ensure the CN implementation is correct and robust before incorporating it into the model. The testing of the CN implementation included the CN algorithm and the iterative solver (Successive over relaxation). FigureD.1 plots the explicit and the CN implicit implementation of the 1D diffusion of a point source (initial concentration = 200 at spatial coordinate 100) at equivalent time points 100, 500 and 1000.

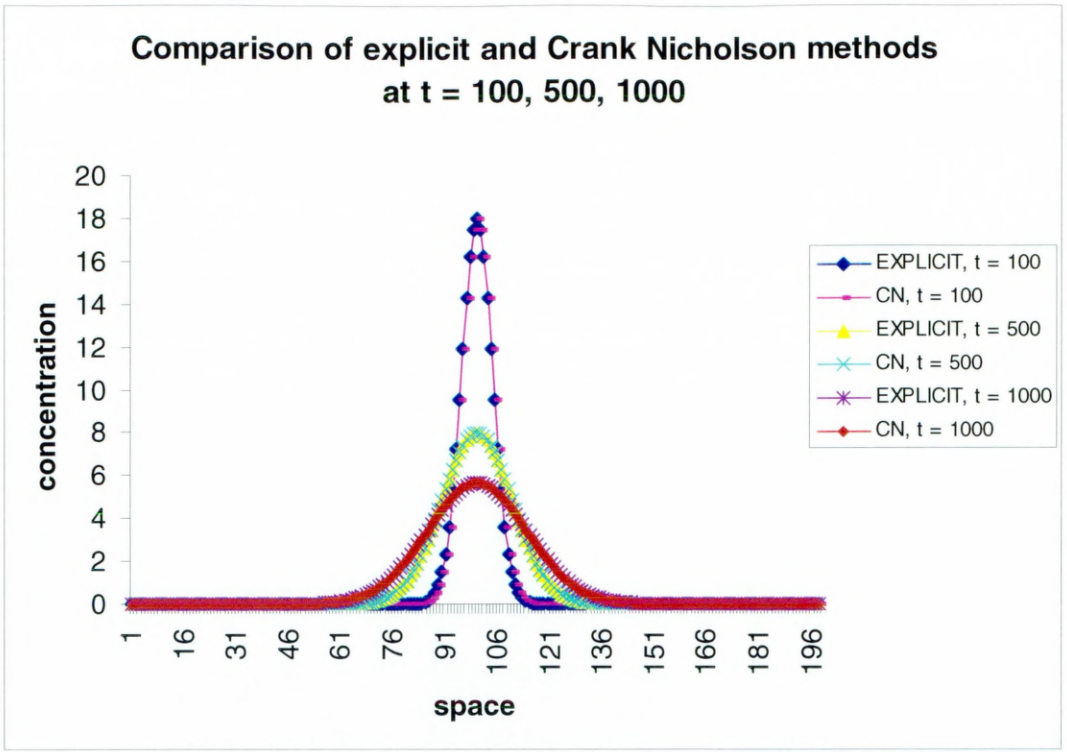


Figure D. 1 Comparison of explicit and implicit numerical scheme

Appendix E. Model Validation and Testing

Overview

As the model was developed testing was undertaken throughout to ensure model validation and verification of implemented algorithms. This allows us to be confident that observed emergent phenomena are not artefacts of imprecise numerical implementations or gross coding errors. These tests are described below:

Testing of numerical algorithms

The implemented numerical algorithms, were tested against the results of an existing numerical implementation or, if possible, an analytical solution. This is carried out to ensure robustness and correctness of the model implementation. The details of verifying the model implementation of the explicit method (FTCS) algorithm is detailed in Section 2.2.1.2 .The details relating to the verification of the Crank Nicholson method are in Appendix D.

Testing of additions using a simple test data set with known desired outcome

When model extensions were added i.e. additional functions each of the functions was tested. Since most functions equated to some mathematical formulae the testing involved a number of scenarios with a range of parameter values. This resulted in plots, for a given parameter set, which were then compared to the corresponding analytical plot. For example the mathematical expression for uptake is a linear product with respect to biomass, external resource and a constant. A plot of how uptake changes with respect to external resource, a known constant and biomass amount is easily determined. Once the uptake function was coded the same parameter values were used as before, and if the formula was implemented correctly the same graph is reproducible. In some cases there were anomalies and debugging was carried out to identify and eliminate the source of error. In addition simple test cases with known inputs and outputs were also used to confirm correctness of implementation. One example of this was diffusion from a point source in a

2D or 3D environment. For a simple diffusive process diffusion of an initial concentration i.e. biomass inoculum should eventually result in a homogeneous uniform concentration over the 2D or 3D environment. This phenomenon was reproducible by the implemented diffusive algorithm.

Conservation of mass

This test was carried out to ensure that conservation of mass was obtained. For a given simulation the quantity of external resource and biomass innocula is known. Resource is subsequently converted into various biomass components, however, the sum of all biomass components and external resource should always be constant, in the absence of replenishment of external resource. If the environment is replenished, it could still be determined by how much, therefore, ensuring conservation of mass. In both cases i.e. with and without replenishment of resource conservation of mass was attained in all simulations.

Appendix F. Published Work

The published paper cited below has been removed from the e-thesis due to copyright restrictions:

Falconer, R.E., Bown, J.L., White, N.A. and Crawford, J.W. (2005). Biomass recycling and the origin of phenotype in fungal mycelia. In *Proceedings of the Royal Society*, 272(1573), pp.1727-1734, doi:10.1098/rspb.2005.3150.